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The final publication is available at:

<https://doi.org/10.1111/pce.13781>

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**Hydraulic and photosynthetic limitations prevail over root non-structural carbohydrate reserves as drivers of resprouting in two Mediterranean oaks**

Journal:	<i>Plant, Cell &amp; Environment</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
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Environment Keywords:	drought, shade
Physiology Keywords:	water relations, storage carbohydrates
Other Keywords:	resprouting, construction costs, survival, growth
Abstract:	<p>Resprouting is an ancestral trait in angiosperms that confers resilience after perturbations. As climate change increases stress, resprouting vigor is declining in many forest regions, but the underlying mechanism is poorly understood. Resprouting in woody plants is thought to be primarily limited by the availability of non-structural carbohydrate reserves (NSC), but hydraulic limitations could also be important. We conducted a multifactorial experiment with two levels of light (ambient, 2-3% of ambient) and three levels of water stress (0, 50 and 80 percent losses of hydraulic conductivity, PLC) on two Mediterranean oaks (<i>Quercus ilex</i> and <i>Q. faginea</i>) under a rain-out shelter (n = 360). The proportion of resprouting individuals after canopy clipping declined</p>

	markedly as PLC increased for both species. NSC concentrations affected the response of <i>Q. ilex</i> , the species with higher leaf construction costs, and its effect depended on the PLC. The growth of resprouting individuals was largely dependent on photosynthetic rates for both species, while stored NSC availability and hydraulic limitations played minor and non-significant roles, respectively. Contrary to conventional wisdom, our results indicate that resprouting in oaks may be primarily driven by complex interactions between hydraulics and carbon sources, whereas stored NSC play a significant but secondary role.

**1 Hydraulic and photosynthetic limitations prevail over root non-structural  
2 carbohydrate reserves as drivers of resprouting in two Mediterranean oaks**

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**Running head:** Hydraulics and photosynthesis limit resprouting

**Funding:** the present study has been supported by the National Natural Science  
Foundation of China (No. 31850410483), the talent funds of Southwest University of  
Science and Technology (No.18ZX7131), the MICINN (AGL2015-69151-R), INIA  
(RTA2015-00054-C02-01) and Generalitat de Catalunya (SGR2017-1518), Spain.

## **Abstract**

Resprouting is an ancestral trait in angiosperms that confers resilience after  
perturbations. As climate change increases stress, resprouting vigor is declining in  
many forest regions, but the underlying mechanism is poorly understood.

Resprouting in woody plants is thought to be primarily limited by the availability of  
non-structural carbohydrate reserves (NSC), but hydraulic limitations could also be  
important. We conducted a multifactorial experiment with two levels of light  
(ambient, 2-3% of ambient) and three levels of water stress (0, 50 and 80 percent  
losses of hydraulic conductivity, PLC) on two Mediterranean oaks (*Quercus ilex* and  
*Q. faginea*) under a rain-out shelter (n = 360). The proportion of resprouting  
individuals after canopy clipping declined markedly as PLC increased for both  
species. NSC concentrations affected the response of *Q. ilex*, the species with higher  
leaf construction costs, and its effect depended on the PLC. The growth of resprouting  
individuals was largely dependent on photosynthetic rates for both species, while

49 stored NSC availability and hydraulic limitations played minor and non-significant  
 50 roles, respectively. Contrary to conventional wisdom, our results indicate that  
 51 resprouting in oaks may be primarily driven by complex interactions between  
 52 hydraulics and carbon sources, whereas stored NSC play a significant but secondary  
 53 role.

54 **Keywords:** construction costs, growth, fire, hydraulics, nonstructural carbohydrate  
 55 concentrations, perturbation, resilience, resprouting vigor, survival, photosynthesis  
 56

57 **Acknowledgements** We acknowledge technical support from MJ Pau, MP Sopeña, A  
 58 Cunill, J Hedó, B Lavaquiol and C Maier.

## 59 **Introduction**

60 Understanding the effects of climatic changes in land cover during this 21<sup>st</sup> century is  
 61 becoming a major research effort globally (IPCC, 2019). Associated with rapid  
 62 climate change, a drying atmosphere and more variable precipitation patterns are  
 63 enhancing water scarcity in many areas of the world. Moreover, as forested  
 64 ecosystems become drier, the prevalence of mega-fires has accelerated, burning  
 65 unprecedented fractions of forested ecosystems (Boer *et al.*, 2020, Nolan *et al.*, 2020).  
 66 In combination with other biotic and abiotic stresses, large scale replacements of  
 67 forests by shrublands or grasslands may become more common (Karavani *et al.*,  
 68 2018).

69       The capacity for resprouting, particularly widespread across angiosperm trees,  
 70 is a major mechanism providing post-fire and post-drought resilience in  
 71 Mediterranean ecosystems and other parts of the world (Sánchez-Pinillos *et al.*,  
 72 2016). However, the capacity for resprouting appears to have limits and is  
 73 diminishing (Karavani *et al.*, 2018), notably with increased drought severity or fire  
 74 recurrence (Batllori *et al.*, 2018, Collins, 2019, Fairman *et al.*, 2019, Pratt *et al.*,  
 75 2014).

76       The mechanisms underlying reductions in the capacity to resprout are still  
 77 relatively unknown. The classic paradigm is that the availability of stored  
 78 carbohydrate reserves is the major driver of resprouting (Bowen & Pate, 1993, Pate *et*  
 79 *al.*, 1990) and that re-sprouting exhaustion would be more likely if carbohydrate  
 80 reserves are reduced, particularly in plants growing under deep shade or that  
 81 experience protracted drought. There is some debate regarding the impact of  
 82 protracted drought on carbohydrate reserves because declines in sink activity under

drought may preclude the decline of source photo-assimilates (Korner, 2003, Palacio *et al.*, 2014).

The role of carbohydrate reserves and plant function has been the topic of considerable research (Dietze *et al.*, 2014). Non-structural carbohydrates (NSC) account for, on average, 10% of dry plant biomass (Martínez-Vilalta *et al.*, 2016). Despite the significant investment into NSC, plant growth is seldom limited by carbon, even under extreme droughts, and NSC depletion is a rare phenomenon in angiosperm trees (Adams *et al.*, 2017, Duan *et al.*, 2013, Martínez-Vilalta *et al.*, 2016, Mitchell *et al.*, 2013). Some authors have proposed that the major function of carbohydrate storage is to serve as a bet-hedging strategy that promotes survival after a perturbation that removes a large part of the aboveground biomass (Wiley & Helliker, 2012).

However, other authors consider that hydraulic limitations developed during drought, either in stems or roots, depending on resprouting type, may impose a higher limitation to post-fire resprouting (Cruz *et al.*, 2003, Karavani *et al.*, 2018). Post-fire resprouting requires new growth, which cannot occur if the capacity for transporting water to meristems is impaired. Additional factors, such as nutrients or bud scarcity, may also limit resprouting (Clarke *et al.*, 2013, Karavani *et al.*, 2018), but the interaction between hydraulics and C starvation (i.e. lack of carbohydrates to support plant function) as drivers of post-fire resprouting is particularly important to resolve under climate change. The frequency of drought-induced defoliation and mortality events are increasing globally (Allen *et al.*, 2015, Carnicer *et al.*, 2011).

Subsequently, fire following a protracted drought could catalyze the conversion of forests to shrublands or grasslands through interactive, but largely unknown, effects on plant hydraulics and carbon balance (Karavani *et al.*, 2018, Parra & Moreno, 2018).



Numerous studies have examined the interactive effects of hydraulics and carbohydrate reserve depletion on drought-induced mortality (Adams *et al.*, 2017), and some studies have addressed fire impacts on xylem vulnerability to embolism in surviving branches (Bar *et al.*, 2019, Hood *et al.*, 2018, Kavanagh *et al.*, 2010, Midgley *et al.*, 2011). However, studies jointly addressing how increasingly larger losses in hydraulic conductance, in addition to increasing levels of carbohydrate reserve depletion, may subsequently limit resprouting are more scarce.

Here, we report the results of an experiment that sought to disentangle the mechanisms affecting resprouting in potted oak seedlings experimentally exposed to three levels of water availability and two levels of light. Treatments were jointly imposed to generate a range in both stored NSC concentrations and losses of hydraulic conductivity.

The oak species (*Quercus ilex* L. and *Quercus faginea* Lam.) in this study are the dominant oaks in the drier parts of the oak expansion range in the Western Mediterranean basin. Regeneration of Mediterranean oaks heavily depends on resprouting because of high seed predation (Herrera, 1995). Understanding the limits to resprouting in these two species is thus important to improve estimates on potential species replacements under climate change. Furthermore, these species show contrasting life history strategies (Palacio *et al.*, 2018): *Q. ilex* is sclerophyllous and evergreen, whereas *Q. faginea* is deciduous and exhibits hydraulic segmentation (Peguero-Pina *et al.*, 2015b).

Overall, we sought to elucidate whether: i) resprouting capacity (percent individuals resprouting) was controlled by hydraulic limitations, NSC limitations (that is, the depletion of NSC reserves) or their interaction; and ii) the growth of the

resprouts exhibited a legacy effect due to previous impacts on hydraulic limitations, reserve availability or concurrent photosynthesis limitations.

We hypothesized that hydraulic limitations would control the percentage of plants that resprout, but subsequent growth of the resprouts would be dependent on NSC content and mobilization to grow the resprouting tissues. Because *Q. ilex* is more scleromorphic than *Q. faginea*, and leaf construction costs (in terms of g glucose g<sup>-1</sup> dry matter) increase with sclerophylly, we expected that resprouting growth would be more dependent on NSC in *Q. ilex* than in *Q. faginea*. Finally, we hypothesized that imposed reductions of light and water availability would decrease concurrent photosynthesis as a source of C, which would additionally limit resprouting. However, we expected that this photosynthesis effect would be minor relative to that of sink (stored NSC) limitations.

## Methods

### *Study site*

The experiment was performed at the experimental field site at the School of Agrifood and Forestry Science and Engineering at the University of Lleida (Spain; 41.62 N, 0.59 E). We constructed a rain-out shelter that was covered by clear polyethylene plastic, as commonly used in greenhouse building. Half of the structure received natural solar radiation, and the other half was covered by dense shade cloth. Plants under ambient light (that is, under the polyethylene plastic) received a maximum PPFD of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and plants under the shade cloth received 2-3% (35-45  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of full sun. The structure had openings on both sides to increase ventilation and minimise heat build-up. The temperature in the rain-out shelter was 3°C higher than outside the shelter under ambient light, and 2.5°C higher in the shade

treatment (Fig. S1). The difference in vapour pressure deficit across sun and shade treatments was 0.1 kPa (Fig. S1)

For this study we obtained 2-year-old seedlings from local nurseries. The provenances originated from the mountain range of the Iberian System. Plants were grown in 11 L pots (20 cm × 20 cm × 27.5 cm). The rooting medium used was the commercial potting mix Humin Substrat N6 (Neihaus, Klasman-Deilmann GmbH, Geeste, Germany). Pots were regularly fertilized with a slow release NPK MgO fertilizer (17-09-11-2, Osmocote Universal, KB, Ecully, France) and daily watered to field capacity until treatment implementation.

The plants grew for 6 months (since February 2017) in the rainout shelter before experiment inception. They developed new leaves under the experimental light conditions, but there may have been legacy effects from the previous growing seasons in the nursery, which could not be quantified. The experiment began in the northern hemisphere summer (July) of 2017 and lasted for 2 years. We induced resprouting by removing the canopy by clipping above the root collar in July 2017, and then again in July 2018 (twice in total; details below).

#### *Experimental treatments*

We induced depletion of carbohydrate reserves and water stress by implementing two different light regimes: sun (ambient) and shade (35-45  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in concert with three different levels of water stress. Light manipulations may alter additional aspects of plant physiology and morphology, but our experimental design was to mimic a comparison between seedlings growing in the understory of a forest or in an open woodland under different levels of water stress.

The three different watering treatments were set to achieve different values of percentage loss of conductivity (PLC); 0, 50 and 80% PLC ( $P_0$ ,  $P_{50}$  and  $P_{80}$ , respectively), which represent different critical water stress thresholds and the potential for recovery in angiosperms (Resco *et al.*, 2009, Urli *et al.*, 2013).  $P_{50}$  represents moderate water stress in angiosperms and  $P_{80}$  represents severe stress, and potential mortality. During treatment implementation, we stopped watering and allowed plants to dehydrate and we measured midday stem water potential ( $\Psi_{md}$ ) every other day in a subset of plants ( $n=5$ ). Stem water potential was measured after introducing the cut stem in zip lock bag, and allowing it to equilibrate for 30 minutes in the dark at room temperature (PMS1000, PMS, Albany, OR). We then calculated the expected PLC based on  $\Psi_{md}$  from previously published vulnerability curves for our species and provenances (Esteso-Martínez *et al.*, 2006, Peguero-Pina *et al.*, 2014). Once plants reached the target PLC, we kept soil moisture constant at that level for two weeks. This was achieved by weighing a subset of pots ( $n=5$  per each treatment) and adding back the water that had evaporated every day. After this 2 weeks period, we conducted the relevant measurements (PLC, predawn water potential ( $\Psi_{pd}$ ) and  $\Psi_{md}$ ) and sampling protocols (for biomass, leaf area, NSC and construction costs) and then induced resprouting. We could not always reach our target levels, but our approach allowed us to establish a gradient of PLCs which was the major goal (see Table 1, S1, Fig. S2). We repeated the same measurements over the two years of the experiment.

Sample size for this experiment was 360 plants: 30 replicates  $\times$  2 species  $\times$  3 water treatments  $\times$  2 light levels. Although all plants were inside the same shelter, we rotated them every fortnight to minimise the impact of microclimatic differences within the shelter on our experimental plants. We measured the basal diameter and

height of all plants at the beginning of the experiment. We used a random number generator to assign plants to treatments. After assigning each plant to a treatment, we ensured there were no significant *a priori* differences in plant size across treatments (ANOVA,  $P > 0.05$ ).

### *Resprouting*

Plants were clipped above the root collar and the pots were then kept at constant weight (to maintain a constant water level) for two more weeks after clipping and, at that point, all pots were regularly watered to field capacity until the next growing season, when the experiment was repeated. That is, we clipped (and sampled) all the 360 plants twice: once in 2017 and then again in 2018. The only exceptions were: i) the plants that died during the experiment as a result of our treatments (i.e. a plant that did not resprout in 2017 was not treated again in 2018); and ii) the plants that were destructively harvested for NSC and construction costs analyses, as detailed below.

We monitored resprouting starting after the first re-watering, two weeks after clipping, by counting the number of resprouting individuals daily for the first two weeks, every two days for the next two weeks, and weekly for the next seven months (no resprouting occurred before re-watering). We quantified resprouting growth by measuring, nine months after clipping, the total length of the re-growing shoots (that is, the sum of the length of all the shoots within a plant), the basal diameter (above the point of clipping) and the volume (calculated from length and diameter assuming the branches formed a cylinder). Throughout the manuscript, resprouting capacity refers to the percentage of individuals that resprouted and resprouting growth relates to changes in length, diameter and volume of resprouting shoots. There was mortality during the experiment as a result of treatment implementation. The percentage of

resprouting individuals was thus calculated relative to the total number of individuals alive (i.e. with stem and green leaves) per treatment before clipping on a given year.

#### *PLC measurements*

We tested whether the watering treatments had reached the target PLC levels by measuring native PLC in 5 plants per treatment. We measured PLC in both experimental years, and always immediately before the clipping. That is, we first measured PLC, then we induced clipping and monitored resprouting twice throughout the experiment (once in each year). We used stem PLC as a proxy of root PLC, an assumption that will be addressed in the discussion.

We measured hydraulic conductivity in stems (50 mm long) before and after removing the air embolisms, as in Sperry *et al.* (1988). We cut the entire shoot, from the root collar, under water in the field. The stem was wrapped in wet paper towels inside zip lock bags in a 4°C cooler and transported to the laboratory. The measured stems were then recut under water once in the laboratory. The procedure used for this study was the same that is detailed in Peguero-Pina *et al.* (2018). Briefly, we perfused distilled, degassed and filtered water (0.22 µm) with 0.005% (v/v) Micropur (Katadyn Products, Wallisellen, Switzerland) to prevent microbial growth. The hydraulic conductivity was measured at a pressure of 0.005 MPa and air embolism was removed at a pressure of 0.15 MPa for 60-90 s. The water flow was measured with a digital mass flowmeter (Liqui-Flow, Bronkhorst High-Tech, Ruurlo, The Netherlands) connected to a computer.

#### *Leaf gas exchange measurements*

We measured leaf gas exchange with a portable photosynthesis system (LI-6400XT, Li-Cor Inc., Lincoln, NE, USA) during the last round of growth measurements on 5 plants per treatment. Measurements were conducted between 10:00h and 14:00h local time, to minimize circadian effects and afternoon stomatal closure. We measured each plant under the two treatment levels of PPFD: 1500 (full sun) and 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (shaded) but here we only report values under growth (treatment) conditions. The other conditions in the chamber were set to 25.0°C, 400 ppm, and 1.5 kPa for temperature, CO<sub>2</sub> and vapour pressure deficit, respectively.

#### *Biomass, leaf area and non-structural carbohydrate analyses*

Immediately prior to clipping, we randomly selected 5 individuals per treatment for destructive measurements. Fresh leaves were first scanned and leaf area and number were calculated with ImageJ for all the leaves in each individual (Schneider *et al.*, 2012). Samples were microwaved quickly after harvest for 30 seconds at 700W, to prevent enzymatic reactions. We then calculated dry biomass by harvesting and weighing the different plant parts (roots, stem, old leaves and new leaves) after drying (48h at 105°C) and calculated leaf mass area (LMA). Roots were manually extracted and washed from the potting mix, carefully to minimize losses of root biomass.

We extracted soluble sugars and starch in roots following Palacio *et al.* (2007). After grinding the dried biomass with a mill (IKA A10, IKA-Werke, Staufen, Denmark), soluble sugars (sugar from now on) and starch were extracted separately. For sugar, we first added 10 ml of ethanol (80% v/v) to 50 mg of sample and waited for 30 minutes at 60°C in a water bath. After this time, we centrifuged (NEYA 8, REMI ELEKTROTECHNIK LTD., Vasai, India) the sample for 10 minutes at 3,200 rpm. We then added 50  $\mu\text{l}$  of supernate, 450  $\mu\text{l}$  of ethanol (80%), 500  $\mu\text{l}$  of phenol

(28%) and 2,500 µl of sulfuric acid (96%), mixed it and let it sit for 30 minutes. The third step was to read the absorbance at 490 nm with the spectrophotometer (Spectrophotometer UV-1600PC, VWR, Radnor, PA, USA) after removing the supernatant and drying the sample at 70°C for 16 hours.

To measure starch content, we added 4 ml of sodium acetate (pH 4.5) to the dry sample and left it for 60 minutes in a 60°C water bath. We then left it to cool and added 1ml of Amyloglucosidase (0.5% w/v), which was then incubated in the stove (16 hours at 50°C). After the incubation, we added 50µl of supernatant to the sample, 450 µl of sodium acetate (pH 4.5), 500µl of phenol (28%) and 2500 µl of sulfuric acid (96%). We then mixed it and let it sit for 30 minutes, we measured again the absorbance at 490 nm with the spectrophotometer. NSC was calculated as the sum of starch and sugars. Unless otherwise noted, NSC refers to the concentration of stored non-structural carbohydrates in the roots.

#### *Construction cost analyses*

We estimated the leaf construction costs following previously developed methods (Nagel *et al.*, 2002, Poorter, 1994, Vertregt & Penning de Vries, 1987). We collected leaves from 5 more individuals per treatment (different to those used in NSC) and we measured ash content after exposing the sample to 400°C for six hours (Nagel *et al.*, 2002). We analysed organic carbon and nitrogen contents as in Aspinwall *et al.* (2017) with a combustion elemental analyser (TruSpec Micro, Leco, St Joseph, MI, USA). Construction costs (CC) were then calculated using the formula from Poorter (1994) as:

$$CC = (-1.041 + 5.077 C) (1-M) + (5.325N) \quad (\text{eq. 1})$$



where M, C and N indicate ash content and organic carbon and nitrogen concentrations, respectively.

### *Data analyses*

We first tested whether our implementation of the water treatments had been successful with linear mixed-effects models (LMM), using  $\Psi_{\text{md}}$  and PLC at the time of inducing resprouting as dependent variables; with light and water treatments as well as species and their interactions as independent variables; and year as a random factor.

We tested for differences in percent resprouting with a generalized linear mixed model (GLMM), using a binomial distribution, where species, light, water treatments and their interactions were fixed factors and year was a random factor. Differences in NSC, construction costs, net assimilation ( $A_{\text{net}}$ ) and growth were analysed with LMM, where species, light, water treatments and their interactions were fixed factors and year was a random factor.

We sought to understand whether resprouting capacity and growth were correlated using GLMM with a binomial distribution, where year was the random factor. In order to understand whether resprouting capacity was limited by hydraulics or by reserves, we used LMM where PLC and NSC and their interactions were the explanatory variables and year was included as a random factor. In order to understand whether growth was driven by hydraulics, reserves or photosynthesis, we additionally used LMM with PLC, NSC and  $A_{\text{net}}$  and their interactions as fixed factors and year as a random factor.

All statistical analyses were implemented in the R software environment (version 3.5.2; R development Core Team, 2018) using the “lme4” package for

GLMM and LMM (Bates *et al.*, 2015), the “car” package for Wald tests in GLMM and LMM (Weisberg, 2019). Additionally, we performed model selection in all of the above cases using automated dredging procedures (Bartoń, 2018). We also calculated the marginal and conditional  $R^2$  ( $R^2_m$  and  $R^2_c$ ), which refer to the fixed or to the random and fixed components in mixed models, respectively, following Nakagawa and Schielzeth (2013).

## Results

### *Water treatment implementation*

Our water treatments were successful in that we managed to create different levels of water stress and, importantly, there were no significant differences across species (Table 1, Table S1, Fig. S1) or across years. However, we could not always reach the desired PLC levels for the following reasons. First, our seedlings were more vulnerable to cavitation than previously published values for the same species (Esteso-Martínez *et al.*, 2006, Peguero-Pina *et al.*, 2014). The value of PLC for a given value of  $\Psi_{md}$  was higher in our seedlings than in published values. Since we measured  $\Psi_{md}$  during our experiment to infer PLC from the published literature, this generated a higher experimental PLC under the P<sub>50\_sun</sub> treatment (between 65 and 68%) than our target value for the oaks (Table 1, Table S1, Fig. S1). The second issue was that our species were very resistant to cavitation and we could not reach the water potentials necessary to induce PLC over 80%, even after leaving the plants without water for two months. Consequently, measured PLC values under the P<sub>80\_sun</sub> treatment (between 69 and 72%) were lower than expected (Table 1, Table S1, Fig. S1). The third issue was that evapotranspiration was low in the shade treatment; consequently, PLC was consistently lower than in the full sun treatment. Indeed, PLC

ranged between 28-41% in the P<sub>50</sub>\_shade treatment, and between 40-54% in the P<sub>80</sub>\_shade treatment (Table 1, Table S1, Fig. S2) in both species. Given this problem P<sub>50</sub>\_shade and P<sub>80</sub>\_shade in the second experimental year were merged under a single treatment (P<sub>50</sub>\_shade).

### *Resprouting capacity*

Resprouting capacity was significantly affected by our experimental treatments. There were differences across species and water levels, and there were also significant interactions between light and water treatments and between species and light treatments (Table 2, Fig. 1).

Taken in isolation, water scarcity had a more important effect on resprouting than light availability: the effect of water was significant ( $P < 0.0001$ ), but light was not significant ( $P > 0.05$ ). Indeed, mean resprouting was 68% in the shade and 63% in the sun (Fig. 1a, b), but resprouting significantly declined with water stress from 88% in P<sub>0</sub>, to 57% in P<sub>50</sub> and to 47% in P<sub>80</sub> (Fig. 1a,c). There was however a significant interaction between the light and water treatments (Fig. 1c). Resprouting in the sun treatment declined from 97% at P<sub>0</sub>, to 62% at P<sub>50</sub> and to 31% at P<sub>80</sub> (Fig. 1c). However, the effect of drought was highly variable in the shade treatment as resprouting varied from 80% in P<sub>0</sub>, to 51% in P<sub>50</sub> and to 79% in P<sub>80</sub> (Fig. 1c).

Regarding the species, *Q. faginea* showed significantly higher (Table 2, Fig. 1a,b) resprouting (77%) than *Q. ilex* (54%). However, species effects depended on the interactions with light (Fig. 1b). Resprouting in *Q. faginea* was favored by the shade, as resprouting increased from 61% in the sun to 95% in the shade (Fig. 1b). However, shade exerted a negative effect on resprouting in *Q. ilex*, which declined from 65% in the sun to 45% in the shade (Fig. 1b).

379

380 *Resprouting growth*

381 Resprouting growth was affected by light, water, and their interaction and, in the case  
 382 of total shoot length, treatment effects depended on the species (Table 2, Fig. S3, Fig.  
 383 2). Total shoot length, basal diameter and shoot volume were always higher in the sun  
 384 than in the shade treatments (Fig. 2, Fig. S3). Total length was higher for *Q. ilex* in  
 385 the shade, but differences across species vanished at high light (Fig. 2a). Total  
 386 resprouting length in the sun declined from 61 cm to 30 cm to 26 cm as water stress  
 387 increased from  $P_0$  to  $P_{50}$  to  $P_{80}$ , respectively (Fig. 2b). Total resprout length in the  
 388 shade showed a less marked decline from 21 cm to 14 cm to 15 cm as water stress  
 389 increased from  $P_0$  to  $P_{50}$  to  $P_{80}$ , respectively (Fig. 2b). A similar pattern was apparent  
 390 for basal diameter, which decreased in the sun from 2.9 mm at  $P_0$  to 2.5 mm at  $P_{50}$  and  
 391 to 2.2 mm in  $P_{80}$  (Fig. 2c). In the shade, however, diameter was higher in  $P_{80}$  (1.85  
 392 mm) than in  $P_0$  (1.4) or  $P_{50}$  (1.5) (Fig. 2c). Differences in volume followed the same  
 393 trend as those in length (Fig. 2d).

394

395 *Coordination between resprouting capacity and resprouting growth*

396 We observed that the patterns of correlation between resprouting capacity and growth  
 397 were highly contrasting across species (Fig. 3). On the one hand, percent resprouting  
 398 in *Q. ilex* correlated positively with total length ( $P < 0.0001$ ,  $R^2_m = 0.28$ ) basal  
 399 diameter ( $P < 0.0001$ ,  $R^2_m = 0.18$ ) and volume ( $P < 0.0001$ ,  $R^2_m = 0.32$ ). On the other  
 400 hand, the correlation between percent resprouting and growth in *Q. faginea* was not  
 401 significant for length ( $P = 0.15$ ) and volume ( $P = 0.5$ ), and negative in the case of  
 402 diameter ( $P < 0.0001$ ), although the power of the negative correlation was very low  
 403 ( $R^2_m = 0.08$ ).

# *Photosynthesis, carbohydrates and construction costs*

Photosynthetic rates measured at ambient light varied across species marginally ( $P < 0.1$ ), across light, water and their interaction significantly ( $P < 0.05$ ) and across the interaction between light and species marginally ( $P < 0.1$ ) (Table 2, Fig. S4). In particular,  $A_{\text{net}}$  in P<sub>0\_sun</sub> treatment for *Q. faginea* ( $13 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was significantly higher than in the P<sub>0\_sun</sub> treatment for *Q. ilex* ( $9.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Average  $A_{\text{net}}$  in all other treatments ranged between  $-0.7$  and  $2.8 \mu\text{mol m}^{-2} \text{s}^{-1}$  but they were not statistically significant, nor was year.

The concentration of root NSC ranged between 7.6-27% in *Q. faginea* and between 8-22% in *Q. ilex*. Consequently, the minimum value of root NSC concentration reached across species was not different (7.6% vs 8.0%) indicating that we reached similar levels of reserve depletion across species. However, *Q. faginea* showed a significantly higher capacity for accumulating NSC on average (18.5% in *Q. faginea* vs 14.2% in *Q. ilex*; Table 2, Fig. S5a).

There were also significant differences in NSC across light treatments (Table 2), with higher concentrations in the sun (20%) relative to the shade (13%) treatment (Fig. S5a, b). However, the effect of light depended on its interaction with the water treatment (Table 2, Fig. S5b). NSC in the shade was highest at the P<sub>80</sub> treatment (15%) and lower at the P<sub>50</sub> (10%) and P<sub>0</sub> (12%) treatments, while in the sun treatments, NSC was lower in the P<sub>80</sub> treatment (18%) compared to the P<sub>50</sub> (19%) and P<sub>0</sub> (22%) treatments. NSC concentrations reflected storage capacity as starch accounted for 71% of the total NSC pool across species. Importantly, year was not significant in the model.

We estimated construction costs on a mass and leaf area basis. On a mass basis, the construction cost of a *Q. faginea* leaf were significantly lower (1.28 g glucose g<sup>-1</sup> dry matter) than for a *Q. ilex* leaf (1.44 g glucose g<sup>-1</sup> dry matter) (Table 2, Fig. S6), indicating that leaf construction costs for *Q. faginea* were 11% lower than for *Q. ilex*. However, the leaf mass area (LMA) of *Q. faginea* (8.8 g cm<sup>-2</sup>) was 39% lower than *Q. ilex* (14.3 g cm<sup>-2</sup>). Consequently, the construction costs of *Q. faginea* on a leaf area basis (0.011 g cm<sup>-2</sup>) were 45% lower than for *Q. ilex* (0.020 g cm<sup>-2</sup>) (Fig. S6a,b). On a leaf area basis, construction costs were also affected by light, water and their interactions with species (Table 2, Fig. S6c, d, e). Leaf construction costs on an area basis were higher in the sun than in the shade (Fig. S6c), and they also increased with drought, particularly in P<sub>80</sub> (Fig. S6d). The interaction between light and water meant that costs in P<sub>80</sub> were constant regardless of light level (Fig. S6e).

#### *Factors affecting resprouting capacity*

We examined the correlation between resprouting capacity and PLC and NSC and we observed different patterns across species (Table 3, Fig. 4). Higher PLC had a negative effect on resprouting, which was significant in *Q. faginea* and *Q. ilex* ( $P < 0.001$ , Table 3). We can examine the point where 50% resprouting occurs, as an analogy with epidemiological studies, which use LD<sub>50</sub> as an indicator of the median lethal dose (or as an analogy with, P<sub>50</sub> broadly used in plant hydraulics). In *Q. faginea*, resprouting was below 50% only when water stress was above P<sub>50</sub> in all cases (Fig. 4b). For *Q. ilex*, resprouting was below 50% only when water stress was above P<sub>50</sub> in all cases except for one point (lower left of Fig. 4a), indicating that NSC limitations may have played an additional role (Fig. 4c).

In *Q. ilex*, there was a significant ( $P > 0.001$ , Table 3, Fig. 4c) and positive correlation between NSC and resprouting capacity, and an interaction between PLC and NSC (Table 3). Resprouting in *Q. faginea* was independent of NSC (Table 3). The  $R^2_m$  of our statistical models were 0.36 for both species (Table 3).

#### *Factors affecting resprouting growth*

Photosynthesis was the most important variable regulating growth of resprouts (Table 3, Fig. 5). The best model included  $A_{net}$  as the only significant parameter for total shoot length in *Q. faginea* ( $P < 0.0001$ ;  $R_m^2 = 0.43$ ) and *Q. ilex* ( $P < 0.0001$ ;  $R_m^2 = 0.76$ ) and for volume in *Q. ilex* ( $P < 0.0001$ ;  $R_m^2 = 0.77$ ). Photosynthesis alone ( $P = 0.001$ ) and, to a minor degree, its interaction with NSC ( $P = 0.01$ ) were significant drivers of volume in *Q. faginea* ( $R_m^2 = 0.56$ ). NSC was an important predictor for diameter in *Q. ilex* ( $P < 0.0001$ ;  $R_m^2 = 0.46$ ), but no variable was a significant predictor of diameter in *Q. faginea* ( $P > 0.05$ ). It is worth noting that PLC did not show a significant correlation with any of the growth parameters (Table 3).

#### **Discussion**

We exposed seedlings of two naturally co-occurring oak species to contrasting light and water conditions for two years to understand the drivers of resprouting. We observed that hydraulic limitations on the capacity to re-sprout were present in both species, but that carbohydrate depletion only affected *Q. ilex*, which was the species with the highest leaf construction costs. Subsequent growth of re-sprouts was primarily driven by current photosynthesis, while stored carbon (NSC) played a significant but secondary role. The mechanisms affecting the capacity for re-sprouting (primarily hydraulics) differed from those affecting the growth of the re-sprouts

(primarily C uptake), although additional differences were observed in two closely related and co-occurring oak species.

#### *Factors affecting resprouting capacity*

The resprouting literature in woody species has traditionally discussed the role of stored carbohydrates and nutrients as the main drivers of re-sprouting (Clarke *et al.*, 2013, Pate *et al.*, 1990). Only a handful of studies have documented water limitations, as indicated by predawn water potential (Cruz *et al.*, 2003), as a potential factor affecting resprouting. However, to the best of our knowledge, this is the first study directly demonstrating that the degree of losses in hydraulic conductance before the perturbation that induces resprouting will limit subsequent resprouting capacity in woody species. Hydraulic limitations were more severe in *Q. faginea* than in *Q. ilex*, even though *Q. faginea* shows a higher degree of hydraulic segmentation (Peguero-Pina *et al.*, 2015a), which is often considered advantageous for recovery after water stress is relieved because runaway cavitation is isolated (Tyree & Zimmerman, 2002).

Our results were only partly consistent with the hypothesis that NSC limits resprouting capacity. We observed a correlation between re-sprouting and NSC only in *Q. ilex*, but the mechanistic reason underlying this relationship remained puzzling after examining differences in leaf construction costs and the amount of reserves, across species. The lowest NSC concentrations in *Q. ilex* roots were 8.1% at the P<sub>50</sub>\_shade treatment which, multiplied by total root weight, adds up to 0.67 g of NSC. The construction costs of a *Q. ilex* leaf at the P<sub>50</sub>\_shade treatment were 0.01 g glucose cm<sup>-2</sup>. These reserves should thus have been enough to build 67 cm<sup>2</sup> of leaves, which is equivalent to 16 leaves in the P<sub>50</sub>\_shade treatment, and to 37% of total leaf area grown within one season. In addition to building new leaves, basal resprouters also



need to rebuild the stem (which has lower construction costs than the leaf, (Poorter, 1994)) and support respiratory costs. However, these numbers indicate that the amount of NSC left would have been enough to resprout and recover canopy photosynthetic capacity.

This apparent contradiction could be partly explained by interactions with hydraulic impairment, which could have limited NSC remobilization (Table 2). *Q. ilex* often showed low re-sprouting (<50%) when NSC and PLC were low (<10%) and high (>50%), respectively. The opposite pattern was also true, as we observed a number of cases in the shade treatment with high re-sprouting rates (>50%) despite NSC being below 10% (Fig. 4c), and this coincided with PLC being <50% (Fig. 4a).

However, and contrary to this hypothesis, PLC was only 20% in the P<sub>50</sub>\_shade treatment for 2018, when re-sprouting was 0% and NSC was 8.1% (Fig. 4a, c). A plausible, but speculative, explanation for this observation is that the root seedlings were disconnected from the soil in the P<sub>50</sub>\_shade treatment. That is, in conditions of low water and shade, the plant may have encountered disruption in the soil-root continuum such that hydraulic constraints occurred, although they could not be picked up by our PLC sampling scheme which focused on main stems. This could also explain why PLC values in the shade were lower than in the sun, also VPD differences across treatments were only minimal. Further studies on the mechanisms addressing resprouting should thus address the disconnection between roots and soils. To our knowledge, this is the first study indicating this could be a possibility.

Differences between *Q. ilex* and *Q. faginea* in resprouting further supports the hypothesis that NSC may only limit resprouting if accompanied by hydraulic impairment. Resprouting in *Q. faginea* was not limited by NSC reserves, although it reached lower NSC concentrations than *Q. ilex*, and this could be because low NSC

did not co-occur with high PLC in *Q. faginea*. That is, the lowest NSC concentrations in *Q. faginea* were 7.6% in the P<sub>50</sub>\_shade treatment, which multiplied by total root weight, equals 0.24 g of NSC. Construction costs were 0.006 g glucose cm<sup>-2</sup>, which indicates that reserves were sufficient to build 40 cm<sup>2</sup> of leaves (or 27% of total leaf area after one growing season). The potential to build new material in *Q. faginea* was thus lower than in *Q. ilex* (which was of 67 cm<sup>2</sup> of leaves, as stated above) because of the lower NSC content. However, we did not observe NSC limitations in this species, which we attribute to the fact that PLC was never above 50% in this species. That is, low NSC alone was not enough to induce reserves limitation in *Q. faginea*. Additional processes, such as bud limitation, could account for some of the explained variation.

#### *Factors affecting resprouting growth*

We observed that resprouting growth was not driven by the same mechanisms as resprouting capacity. One of the differences was that hydraulic limitations developed before the perturbation did not exert any carry-over effects over the growth of the resprouts. This contrasts with drought responses, where hydraulics have been documented to affect plant growth for some years after the drought (Anderegg *et al.*, 2015, Ogle *et al.*, 2015). This difference is likely driven by the fact that we focused on basal resprouting, where new tissue that is free from embolisms is grown. We thus cannot discard a role for hydraulics limiting the growth of epicormic sprouts, particularly for species that maintain their xylem functional for many years. We note that one could have encountered drought legacies if the period between clipping and re-watering had been longer than in this experiment.

Another difference between the drivers of re-sprouting growth and capacity was that net photosynthesis appeared to correlate best with growth. Photosynthesis

was the sole correlate of height in both species and of volume in *Q. ilex*, indicating that source rather than sink limitations control resprouting in these species. This is perhaps not surprising considering the strong reductions in C uptake imposed by both our shading and drought treatments.

The potential role of NSC as a driver of growth was overall weak. NSC correlated with secondary growth in *Q. ilex* and, along with photosynthesis, it affected volume in *Q. faginea*. Such a weak role of NSC may be puzzling, considering the wealth of literature supporting a role of carbohydrates as major drivers of re-sprouting (Pausas & Keeley, 2014, Zeppel *et al.*, 2015). However, our results are consistent with the drought literature, where overall, NSC concentrations seldom limit plant survival or growth in angiosperms (Adams *et al.*, 2017, Korner, 2003).

Our results indicate that source limitations to growth were the most common under the different stresses, but sink processes also played a role. Following (and simplifying) the model from Huang *et al.* (2019) and Gessler and Grossiord (2019), under source limitation we should observe that photosynthetic declines lead to a decline in root reserves. This pattern of source limitation is consistent with our results in the shade treatment, where photosynthetic rates and root NSC concentrations were lower than in the P<sub>0</sub>\_sun. Our temporal design does not allow us to determine the precise moment when NSC concentrations declined, but most likely it occurred during the first few months after treatment implementation because we did not observe differences across years at the moment prior to clipping.

Similarly, there were significant declines in NSC concentration as water stress increased in the sun treatments, further indicating source limitations to growth in the sun across drought treatments. However, we observed that NSC concentrations in P<sub>80</sub> treatments were higher than in P<sub>0</sub> treatments in the shade, a pattern that is consistent

with sink limitations. That is, Huang *et al.* (2019) and Gessler and Grossiord (2019) propose that when sink limitations occur, a decline in root metabolic activity leads to an increase in metabolite accumulation. This pattern was consistent with the results obtained after imposing the double stress of shade and drought, indicating that multiple stress responses and metabolic limitations co-occur.

The evolutionary role of NSC reserves requires further assessment since NSC constituted significant portions of plant dry mass in the roots and yet exhibited a limited role in regulating re-sprouting capacity and primary growth.

#### *Limitations on the use of stem PLC as a proxy for root PLC*

We need to acknowledge that a limitation of this study was the use of stem PLC as a proxy for root PLC. Previous studies have indicated how this proxy can be problematic when there is substantial hydraulic segmentation (Johnson *et al.*, 2016) because roots have sometimes (Tyree & Zimmerman, 2002), but not always (Wu *et al.*, 2020), been documented to be more vulnerable to embolism than stems. Although the existence of hydraulic segmentation was formulated long ago (Zimmermann, 1978), it is still unclear how prevalent it is across species (Wu *et al.*, 2020).

For the two species of concern here, we could not find any study addressing whether hydraulic segmentation occurs in seedlings. Previous studies conducted with adult trees (Peguero-Pina *et al.*, 2015a), however, observed that leaves of *Q. subpyrenaica* (the hybrid between *Q. faginea* and *Q. pubescens*) withered prematurely as leaf water potentials dropped below -3MPa, which indicates hydraulic segmentation between leaves and stems. The same study did not find vulnerability segmentation between leaves and stems in *Q. ilex*.

For adult trees of *Q. ilex*, (Martínez-Vilalta *et al.*, 2002) documented that roots were more vulnerable to cavitation than stems. The differences in the relationship between applied pressure and PLC across tissues were particularly high as PLC increased above 50%. That is, while there were no statistical differences in the  $P_{50}$  of roots and stems, roots became increasingly more vulnerable to cavitation than stems at higher water potentials (Martínez-Vilalta *et al.*, 2002).

We thus cannot discard the possibility that root PLC in the  $P_{80\_sun}$  treatment for *Q. ilex* would have been larger than the one measured for stem PLC. A similar response may also have occurred in *Q. faginea*, if this species also displays vulnerability segmentation between roots and stems. That is, stem PLC may have underestimated root PLC, and the differences in PLC across organs would have been more accentuated at higher PLCs. If this is the case, we can speculate that the slope of the relationship between PLC and resprouting capacity would have been steeper than reported here (Fig. 4, Table 3).

We must acknowledge also the possibility that stem PLC in our species is not indicative of root PLC at all. Under this scenario, our results would indicate that stem PLC before clipping, and not root PLC, controls basal resprouting after clipping. However, there is no known mechanism to explain this relationship and we thus consider it unlikely.

### *Quercus resilience*

Overall, our results indicated that oak seedlings were more resilient to clipping in the shade than in the sun. That is, light and water limitations (shade and drought) induced a higher level of resprouting than water limitations (drought) under no light limitation (sun). However, there were differences in PLC across treatments, which were

substantially higher in the sun than in the shade. Indeed, comparisons on drought responses across different light treatments were affected by the different severities of drought. It should thus be noted that in the field, shady sites may sometimes be drier than sunny sites because of increased plant-plant competition for water (Valladares *et al.*, 2016). Under these circumstances, when shady environments are drier than sunny sites, we would thus expect a lower re-sprouting capacity in the shade than in the sun.

Previous studies assessing the recovery from drought in angiosperms indicate that lack of recovery may occur when PLC>80% (Resco *et al.*, 2009, Urli *et al.*, 2013). Our results on this regard are still tentative, and limited as they are based on stem PLC (and not root PLC), but they allow us to hypothesize that re-sprouting may become particularly limited when PLC is below 50%. Hence, resprouters are more susceptible to stress and disturbance, than to stress alone. It should be noted that we induced resprouting by clipping, to ensure homogeneity in treatments, but fire exerts an additional thermal stress (Casals & Rios, 2018). It is thus feasible that the hypothesized PLC threshold of 50% for limiting resprouting may be lower in burned plants.

It should also be noted that our results are limited to basal resprouting and further studies will need to address potential limitations in epicormic and other types of resprouting. In particular, the role of hydraulic legacies may be more important in epicormic resprouters. There are an increasing number of studies indicating increased intensity of forest fires under global warming, resulting from higher temperatures and drought intensities (Boer *et al.*, 2016, Jolly *et al.*, 2015, Nolan *et al.*, 2020). Our results offer new mechanistic insights and hypotheses to be tested in further experiments that may be used to improve model predictions. We thus hope this study

serves as a starting point for more studies on this area to further clarify outstanding issues.

## **Acknowledgements**

The present study has been supported by the National Natural Science Foundation of China (No. 31850410483), the talent funds of Southwest University of Science and Technology (No.18ZX7131), the MICINN (AGL2015-69151-R), INIA (RTA2015-00054-C02-01) and Generalitat de Catalunya (SGR2017-1518), Spain. We acknowledge technical support from MJ Pau, MP Sopeña, A Cunill, J Hedo, B Lavaquiol and C Maier.

## **Authorship statement**

VRD conceived the project with input from CA, JJPP, DSK, WS, DGW, MMB, JV, JMM, DTT and EGP; CA, EGP, JJPP and DSK cultivated the plants; CA, VRD, EGP, JJPP, DSK, OKZ, DTT and HQ performed measurements; CA prepared the data files; VRD analyzed the data and wrote the manuscript and all co-authors contributed to edits to the manuscript.

## **Conflict of interest**

The authors declare no conflict of interest

## **References**

Adams H.D., Zeppel M.J.B., Anderegg W.R.L., Hartmann H., Landhäusser S.M., Tissue D.T., . . . McDowell N.G. (2017) A multi-species synthesis of

- 675 physiological mechanisms in drought-induced tree mortality. *Nature Ecology*  
 676 *& Evolution*, **1**, 1285-1291.
- 677 Allen C.D., Breshears D.D. & McDowell N.G. (2015) On underestimation of global  
 678 vulnerability to tree mortality and forest die-off from hotter drought in the  
 679 Anthropocene. *Ecosphere*, **6**, 129.
- 680 Anderegg W.R.L., Schwalm C., Biondi F., Camarero J.J., Koch G., Litvak M., . . .  
 681 Pacala S. (2015) Pervasive drought legacies in forest ecosystems and their  
 682 implications for carbon cycle models. *Science*, **349**, 528-532.
- 683 Aspinwall M.J., Varhammar A., Blackman C.J., Tjoelker M.G., Ahrens C., Byrne M.,  
 684 . . . Rymer P.D. (2017) Adaptation and acclimation both influence  
 685 photosynthetic and respiratory temperature responses in *Corymbia calophylla*.  
 686 *Tree Physiol*, **37**, 1095-1112.
- 687 Bar A., Michaletz S.T. & Mayr S. (2019) Fire effects on tree physiology. *New Phytol*.
- 688 Bartoń K. (2018) MuMIn: Multi-Model Inference. R package version 1.42.1.  
 689 <https://CRAN.R-project.org/package=MuMIn>.
- 690 Bates D., Maechler M., Bolker B. & Walker S. (2015) Fitting Linear Mixed-Effects  
 691 Models using lme4. *Journal of Statistical Software*, **67**, 1-48.
- 692 Batllori E., De Cáceres M., Brotons L., Ackerly D.D., Moritz M.A., Lloret F. &  
 693 Oliveras I. (2018) Compound fire-drought regimes promote ecosystem  
 694 transitions in Mediterranean ecosystems. *Journal of Ecology*, **107**, 1187-1198.
- 695 Boer M.M., Bowman D.M.J.S., Murphy B.P., Cary G.J., Cochrane M.A., Fensham  
 696 R.J., . . . Bradstock R.A. (2016) Future changes in climatic water balance  
 697 determine potential for transformational shifts in Australian fire regimes.  
 698 *Environ Res Let*, **11**, 065002.



- 699 Boer M.M., Resco de Dios V. & Bradstock R.A. (2020) Unprecedented burn area of  
 700 Australian mega forest fires. *Nature Clim Change*, **10**, 171-172.
- 701 Bowen B.J. & Pate J.S. (1993) The significance of root starch in postfire shoot  
 702 recovery of the resprouter *Stirlingia latifolia* R. Br. (Proteaceae). *Ann. Bot.*, **72**,  
 703 7-16.
- 704 Carnicer J., Coll M., Ninyerola M., Pons X., Sánchez G. & Peñuelas J. (2011)  
 705 Widespread crown condition decline, food web disruption, and amplified tree  
 706 mortality with increased climate change-type drought. *Proceedings of the*  
 707 *National Academy of Sciences*, **108**, 1474-1478.
- 708 Casals P. & Rios A.I. (2018) Burning intensity and low light availability reduce  
 709 resprouting ability and vigor of *Buxus sempervirens* L. after clearing. *Sci*  
 710 *Total Environ.*, **627**, 403-416.
- 711 Clarke P.J., Lawes M.J., Midgley J.J., Lamont B.B., Ojeda F., Burrows G.E., . . .  
 712 Knox K.J.E. (2013) Resprouting as a key functional trait: how buds, protection  
 713 and resources drive persistence after fire. *New Phytol.*, **197**, 19-35.
- 714 Collins L. (2019) Eucalypt forests dominated by epicormic resprouters are resilient to  
 715 repeated canopy fires. *Journal of Ecology*, **108**, 310-324.
- 716 Cruz A., Pérez B. & Moreno J.M. (2003) Plant stored reserves do not drive  
 717 resprouting of the lignotuberous shrub *Erica australis*. *New Phytologist*, **157**,  
 718 251-261.
- 719 Dietze M.C., Sala A., Carbone M.S., Czimczik C.I., Mantoosh J.A., Richardson A.D.  
 720 & Vargas R. (2014) Nonstructural carbon in woody plants. *Annu Rev Plant*  
 721 *Biol.*, **65**, 667-687.

- 722 Duan H., Amthor J.S., Duursma R.A., O'Grady A.P., Choat B. & Tissue D.T. (2013)  
 723 Carbon dynamics of eucalypt seedlings exposed to progressive drought in  
 724 elevated [CO<sub>2</sub>] and elevated temperature. *Tree Physiol*, **33**, 779-792.
- 725 Esteso-Martínez J., Camarero J.J. & Gil-Pelegrín E. (2006) Competitive effects of  
 726 herbs on *Quercus faginea* seedlings inferred from vulnerability curves and  
 727 spatial-pattern analyses in a Mediterranean stand (Iberian System, northeast  
 728 Spain). *Écoscience*, **13**, 378-387.
- 729 Fairman T.A., Bennett L.T. & Nitschke C.R. (2019) Short-interval wildfires increase  
 730 likelihood of resprouting failure in fire-tolerant trees. *J Environ Manage*, **231**,  
 731 59-65.
- 732 Gessler A. & Grossiord C. (2019) Coordinating supply and demand: plant carbon  
 733 allocation strategy ensuring survival in the long run. *New Phytol*, **222**, 5-7.
- 734 Herrera J. (1995) Acorn predation and seedling production in a low-density  
 735 population of cork oak (*Quercus suber* L.). *Forest Ecology and Management*,  
 736 **76**, 197-201.
- 737 Hood S.M., Varner J.M., van Mantgem P. & Cansler C.A. (2018) Fire and tree death:  
 738 understanding and improving modeling of fire-induced tree mortality.  
 739 *Environmental Research Letters*, **13**, 113004.
- 740 Huang J., Hammerbacher A., Weinhold A., Reichelt M., Gleixner G., Behrendt T., . . .  
 741 Hartmann H. (2019) Eyes on the future - evidence for trade-offs between  
 742 growth, storage and defense in Norway spruce. *New Phytol*, **222**, 144-158.
- 743 IPCC (2019) *Climate Change and Land: an IPCC special report on climate change,*  
 744 *desertification, land degradation, sustainable land management, food security,*  
 745 *and greenhouse gas fluxes in terrestrial ecosystems.*

- 746 Johnson D.M., Wortemann R., McCulloh K.A., Jordan-Meille L., Ward E., Warren  
 747 J.M., . . . Domec J.C. (2016) A test of the hydraulic vulnerability segmentation  
 748 hypothesis in angiosperm and conifer tree species. *Tree Physiol*, **36**, 983-993.
- 749 Jolly W.M., Cochrane M.A., Freeborn P.H., Holden Z.A., Brown T.J., Williamson  
 750 G.J. & Bowman D.M.J.S. (2015) Climate-induced variations in global wildfire  
 751 danger from 1979 to 2013. *Nature Communications*, **6**, 7537.
- 752 Karavani A., Boer M.M., Baudena M., Colinas C., Díaz-Sierra R., Pemán J., . . .  
 753 Resco de Dios V. (2018) Fire-induced deforestation in drought-prone  
 754 Mediterranean forests: drivers and unknowns from leaves to communities.  
 755 *Ecological Monographs*, **88**, 141-169.
- 756 Kavanagh K.L., Dickinson M.B. & Bova A.S. (2010) A way forward for fire-caused  
 757 tree mortality prediction: Modeling a physiological consequence of fire. *Fire*  
 758 *Ecol*, **6**, 80-94.
- 759 Korner C. (2003) Carbon limitation in trees. *Journal of Ecology*, **91**, 4-17.
- 760 Martínez-Vilalta J., Prat E., Oliveras I. & Piñol J. (2002) Xylem hydraulic properties  
 761 of roots and stems of nine Mediterranean woody species. *Oecologia*, **133**, 19-  
 762 29.
- 763 Martínez-Vilalta J., Sala A., Asensio D., Galiano L., Hoch G., Palacio S., . . . Lloret  
 764 F. (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a  
 765 global synthesis. *Ecological Monographs*, **86**, 495-516.
- 766 Midgley J.J., Kruger L.M. & Skelton R. (2011) How do fires kill plants? The  
 767 hydraulic death hypothesis and Cape Proteaceae “fire-resisters”. *South Afr J*  
 768 *Bot*, **77**, 381-386.
- 769 Mitchell P.J., O'Grady A.P., Tissue D.T., White D.A., Ottenschlaeger M.L. & Pinkard  
 770 E.A. (2013) Drought response strategies define the relative contributions of

- 771 hydraulic dysfunction and carbohydrate depletion during tree mortality. *New*  
 772 *Phytol*, **197**, 862-872.
- 773 Nagel J.M., Griffin K.L., Schuster W.S., Tissue D.T., Turnbull M.H., Brown K.J. &  
 774 Whitehead D. (2002) Energy investment in leaves of red maple and co-  
 775 occurring oaks within a forested watershed. *Tree Physiol*, **22**, 859-867.
- 776 Nakagawa S. & Schielzeth H. (2013) A general and simple method for obtaining  $R^2$   
 777 from generalized linear mixed-effects models. *Methods Ecol Evol*, **4**, 133-142.
- 778 Nolan R.H., Boer M.M., Collins L., Resco de Dios V., Clarke H., Jenkins M., . . .  
 779 Bradstock R.A. (2020) Causes and consequences of eastern Australia's 2019–  
 780 20 season of mega-fires. *Global Change Biology*, **26**, 1039-1041.
- 781 Ogle K., Barber J.J., Barron-Gafford G.A., Bentley L.P., Young J.M., Huxman T.E., .  
 782 . . Tissue D.T. (2015) Quantifying ecological memory in plant and ecosystem  
 783 processes. *Ecol Lett*, **18**, 221-235.
- 784 Palacio S., Camarero J.J., Maestro M., Alla A.Q., Lahoz E. & Montserrat-Martí G.  
 785 (2018) Are storage and tree growth related? Seasonal nutrient and  
 786 carbohydrate dynamics in evergreen and deciduous Mediterranean oaks.  
 787 *Trees*, **32**, 777-790.
- 788 Palacio S., Hoch G., Sala A., Körner C. & Millard P. (2014) Does carbon storage  
 789 limit tree growth? *New Phytol*, **201**, 1096-1100.
- 790 Palacio S., Maestro M. & Montserrat-Marti G. (2007) Relationship between shoot-  
 791 rooting and root-sprouting abilities and the carbohydrate and nitrogen reserves  
 792 of Mediterranean dwarf shrubs. *Ann Bot*, **100**, 865-874.
- 793 Parra A. & Moreno J.M. (2018) Drought differentially affects the post-fire dynamics  
 794 of seeders and resprouters in a Mediterranean shrubland. *Science of the Total*  
 795 *Environment*, **626**, 1219-1229.

- 796 Pate J.S., Froend R.H., Bowen B.J., Hansen A. & Kuo J. (1990) Seedling growth and  
 797 storage characteristics of seeder and resprouter species of Mediterranean-type  
 798 ecosystems of S.W. Australia. . *Ann Bot*, **65**, 585-601.
- 799 Pausas J.G. & Keeley J.E. (2014) Evolutionary ecology of resprouting and seeding in  
 800 fire-prone ecosystems. *New Phytologist*, **204**, 55-65.
- 801 Peguero-Pina J.J., Mendoza-Herrer O.s., Gil-Pelegrín E. & Sancho-Knapik D. (2018)  
 802 Cavitation limits the recovery of gas exchange after severe drought stress in  
 803 Holm Oak (*Quercus ilex* L.). *Forests*, **9**, 443.
- 804 Peguero-Pina J.J., Sancho-Knapik D., Barron E., Camarero J.J., Vilagrosa A. & Gil-  
 805 Pelegrin E. (2014) Morphological and physiological divergences within  
 806 *Quercus ilex* support the existence of different ecotypes depending on climatic  
 807 dryness. *Ann Bot*, **114**, 301-313.
- 808 Peguero-Pina J.J., Sancho-Knapik D., Martín P., Saz M.Á., Gea-Izquierdo G.,  
 809 Cañellas I. & Gil-Pelegrín E. (2015a) Evidence of vulnerability segmentation  
 810 in a deciduous Mediterranean oak (*Quercus subpyrenaica* E. H. del Villar).  
 811 *Trees*, **29**, 1917-1927.
- 812 Peguero-Pina J.J., Siso S., Sancho-Knapik D., Diaz-Espejo A., Flexas J., Galmes J. &  
 813 Gil-Pelegrin E. (2015b) Leaf morphological and physiological adaptations of a  
 814 deciduous oak (*Quercus faginea* Lam.) to the Mediterranean climate: a  
 815 comparison with a closely related temperate species (*Quercus robur* L.). *Tree*  
 816 *Physiol.*
- 817 Poorter H. (1994) Construction costs and payback time of biomass: a whole plant  
 818 approach. In: *A whole plant perspective on carbon-nitrogen interactions*. (eds  
 819 J. Roy & J. Garnier), pp. 111-127. Backhuys Publishers, Leiden.

- 820 Pratt R.B., Jacobsen A.L., Ramirez A.R., Helms A.M., Traugh C.A., Tobin M.F., . . .  
 821 Davis S.D. (2014) Mortality of resprouting chaparral shrubs after a fire and  
 822 during a record drought: physiological mechanisms and demographic  
 823 consequences. *Glob Chang Biol*, **20**, 893-907.
- 824 Resco V., Ewers B.E., Sun W., Huxman T.E., Weltzin J.F. & Williams D.G. (2009)  
 825 Drought-induced hydraulic limitations constrain leaf gas exchange recovery  
 826 after precipitation pulses in the C<sub>3</sub> woody legume, *Prosopis velutina*. *New*  
 827 *Phytologist*, **181**, 672-682.
- 828 Sánchez-Pinillos M., Coll L., De Cáceres M. & Ameztegui A. (2016) Assessing the  
 829 persistence capacity of communities facing natural disturbances on the basis  
 830 of species response traits. *Ecological Indicators*, **66**, 76-85.
- 831 Schneider C.A., Rasband W.S. & Eliceiri K.W. (2012) NIH Image to ImageJ: 25  
 832 years of image analysis. *Nature methods*, **9**, 671-675.
- 833 Sperry J.S., Donnelly J.R. & Tyree M.T. (1988) A method for measuring hydraulic  
 834 conductivity and embolism in xylem. *Plant, Cell and Environment*, **11**, 35-40.
- 835 Tyree M.T. & Zimmerman M.H. (2002) *Xylem structure and the ascent of sap*. (2nd  
 836 ed.). Springer, Verlin.
- 837 Urli M., Porté A.J., Cochard H., Guengant Y., Burlett R. & Delzon S. (2013) Xylem  
 838 embolism threshold for catastrophic hydraulic failure in angiosperm trees.  
 839 *Tree Physiol*, **33**, 672-683.
- 840 Valladares F., Laanisto L., Niinemets Ü. & Zavala M.A. (2016) Shedding light on  
 841 shade: ecological perspectives of understorey plant life. *Plant Ecology &*  
 842 *Diversity*, **9**, 237-251.
- 843 Vertregt N. & Penning de Vries F.W.T. (1987) A rapid method for determining the  
 844 efficiency of biosynthesis of plant biomass. *J Theor Biol*, **128**, 109-119.

- 845 Wiley E. & Helliker B. (2012) A re-evaluation of carbon storage in trees lends greater  
 846 support for carbon limitation to growth. *New Phytol*, **195**, 285-289.
- 847 Wu M., Zhang Y., Oya T., Marcati C.R., Pereira L. & Jansen S. (2020) Root xylem in  
 848 three woody angiosperm species is not more vulnerable to embolism than stem  
 849 xylem. *Plant and Soil*.
- 850 Zeppel M.J.B., Harrison S.P., Adams H.D., Kelley D.I., Li G., Tissue D.T., . . .  
 851 McDowell N.G. (2015) Drought and resprouting plants. *New Phytologist*, **206**,  
 852 583-589.
- 853 Zimmermann M.H. (1978) Hydraulic architecture of some diffuse-porous trees. *Can J*  
 854 *Bot*, **56**, 2286-2295.

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# 858 **Supporting information**

859 **Table S1:** results of linear mixed models analyses on differences across realized PLC  
 860 values. Fixed factors were species, light and water treatments and their interactions  
 861 and year was included as a random factor.

862

863 **Figure S1:** Differences in mean daily air temperature ( $T_{\text{air}}$ ) and mean daily vapour  
 864 pressure deficit (VPD) between sun and shade treatments (a, b) and in  $T_{\text{air}}$  between  
 865 treatments and ambient (c,d). Data in the shelters were collected between June and  
 866 July 2017 with a PCE-HT 71N (PCE Ibérica, Tobarra, Spain) logging automatically  
 867 every 5 minutes. Ambient  $T_{\text{air}}$  data was downloaded from station number 9771C, from  
 868 the Spanish Meteorology Agency ([www.aemet.es](http://www.aemet.es), 21<sup>st</sup> February 2020), which is placed  
 869 inside the campus (<1 Km from the shelter).

870

871 **Fig. S2:** Realized values of percent loss of hydraulic conductance (PLC) and midday  
 872 water potential ( $\Psi_{md}$ ) at the time of inducing resprouting in *Q. ilex* (a,b) and *Q.*  
 873 *faginea* (c,d). The target values were 0, 50 and 80% PLC ( $P_0$ ,  $P_{50}$  and  $P_{80}$ ,  
 874 respectively). Values indicate mean and standard error.

875

876 **Fig. S3:** Changes in resprouting growth as a function of species, water and light  
 877 levels. The species are *Quercus ilex* (Qi) and *Q. faginea* (Qf). The water levels refer  
 878 to target levels of PLC ( $P_0$ ,  $P_{50}$  or  $P_{80}$ , but see Table 1 for actual values). Sun or shade  
 879 indicate whether the plants received either ambient, or 2-3% of ambient radiation,  
 880 respectively. Values indicate mean and standard error.

881

882 **Fig. S4:** Changes in net assimilation rate across species, light and water treatments.  
 883 To more clearly depict significant effects, we only include significant interactions  
 884 (but note that the interaction in c is marginal). The water levels refer to target levels of  
 885 PLC ( $P_0$ ,  $P_{50}$  or  $P_{80}$ , but see Table1 for actual values). Sun or shade indicate whether  
 886 the plants received either ambient, or 2-3% of ambient radiation, respectively. Values  
 887 indicate mean and standard error.

888

889 **Fig. S5:** Changes in non structural carbohydrates (NSC) across species, light and  
 890 water treatments. To more clearly depict significant effects, we only include  
 891 significant interactions. The water levels refer to target levels of PLC ( $P_0$ ,  $P_{50}$  or  $P_{80}$ ,  
 892 but see Table1 for actual values). Sun or shade indicate whether the plants received  
 893 either ambient, or 2-3% of ambient radiation, respectively. Values indicate mean and  
 894 standard error.



895

896 **Fig. S6:** Construction costs on a mass basis (a) and on a leaf area basis (b-e) as a  
897 function of experimental treatments. To more clearly depict significant effects, we  
898 only include significant interactions. The water levels refer to target levels of PLC  
899 ( $P_0$ ,  $P_{50}$  or  $P_{80}$ , but see Table 1 for actual values). Sun or shade indicate whether the  
900 plants received either ambient, or 2-3% of ambient radiation, respectively. Values  
901 indicate mean and standard error.

902

**Table 1:** Target midday water potential ( $\Psi_{md}$ ) to reach the desired PLC according to Estes-Martínez *et al.* (2006) for *Q. faginea* and to Peguero-Pina *et al.* (2014) for *Q. ilex*, and actual values. Mean (and SE) actual values are presented. The letters in “Actual PLC” indicate the results of post-hoc analyses (Tukey HSD).

Species	Light treatment	Water treatment	Target $\Psi_{md}$ (MPa)	Target PLC (%)	Actual $\Psi_{md}$ (MPa)	Actual PLC (%)
<i>Q. ilex</i>	Sun	P <sub>0</sub>	> -4	< 20	-1.3 (0.20)	11.9 (2.3) <sup>c</sup>
		P <sub>50</sub>	-6	50	-5.4 (0.54)	64.7 (6.87) <sup>a</sup>
		P <sub>80</sub>	-8.9	80	-6.4 (0.62)	69.1 (7.56) <sup>ab</sup>
	Shade	P <sub>0</sub>	> -4	< 20	-0.8 (0.14)	12.3 (2.84) <sup>c</sup>
		P <sub>50</sub>	-6	50	-2.9 (0.77)	41.2 (10.9) <sup>abc</sup>
		P <sub>80</sub>	-8.9	80	-4.4 (0.56)	54.3 (10.69) <sup>ab</sup>
<i>Q. faginea</i>	Sun	P <sub>0</sub>	> -2.8	< 20	-1.1 (0.22)	15.6 (2.73) <sup>c</sup>
		P <sub>50</sub>	-3.9	50	-3.9 (0.28)	53.2 (8.04) <sup>ab</sup>
		P <sub>80</sub>	-5.5	80	-4.2 (0.30)	66.1 (9.89) <sup>a</sup>
	Shade	P <sub>0</sub>	> -2.8	< 20	-0.7 (0.12)	7.5 (1.35) <sup>c</sup>
		P <sub>50</sub>	-3.9	50	-1.1 (0.21)	27.9 (9.86) <sup>bc</sup>
		P <sub>80</sub>	-5.5	80	-2.6 (0.51)	39.8 (13.0) <sup>abc</sup>

**Table 2:** Results of a fitting a generalized linear mixed effects model (GLMM) on resprouting capacity, a linear mixed effects model (LMM) on length, diameter, volume, net assimilation ( $A_{\text{net}}$ ) and non-structural carbohydrate concentrations (NSC), and an ANOVA on leaf construction costs on a mass (CC) and an area (CC\_area) basis. The explanatory variables were in all cases species, light, water and their two- and three-way interactions included. We applied dredging techniques for automatic model selection and only the variables selected are included. Year was included as a random factor in GLMM and LMM models. ANOVA was performed for CC and CC\_area because only data for 2018 was available. Values indicate either the  $\chi^2$  (GLMM and LMM) or the F-values (ANOVA) and stars indicate the significance level.

	Resprouting capacity	Length	Diameter	Volume	$A_{\text{net}}$	NSC	CC	CC_area
Species	26.6***				3.1	9.58**	46.3***	1299***
Light		157***	155***	154.9***	108***	50.1***		165.1***
Water	86***	67.7***	43.8***	43.9***	91.7***			699.5***
Light $\times$ Water	64.4***	34.2***	52.9***	52.9***	88.7***	0.01**		206.6***
Species $\times$ Light	67.4***	7.8**			3.2			67.7***
Species $\times$ Water								24.7***
Species $\times$ Water $\times$ Light								38.6***

Significance levels: \*\*\* <0.001; \*\*<0.01; \*<0.05; ·<0.1.

**Table 3:** Effects of PLC and NSC over resprouting capacity and additionally of net photosynthesis ( $A_{\text{net}}$ ) over shoot total length, basal diameter and volume. Resprouting capacity was fitted with generalized additive mixed model (GAMM) and growth with a linear mixed-effects model (LMM). We applied dredging techniques for automatic model selection and only the variables selected in the best model are included. Values indicate the  $\chi^2$  and stars indicate the significance level. Values are also provided for the marginal  $R^2$  ( $R^2_m$ ) for each model.

	Resprouting capacity	Length	Diameter	Volume
<i>Q. ilex</i> ;	$R^2_m = 0.36$	$R^2_m = 0.76$	$R^2_m = 0.46$	$R^2_m = 0.77$
PLC	43.2***			
NSC	29.2***		16.7***	
PLC $\times$ NSC	14.9***			
$A_{\text{net}}$		34.5***		25.9***
<i>Q. faginea</i>	$R^2_m = 0.36$	$R^2_m = 0.43$		$R^2_m = 0.56$
PLC	92.8***			
NSC				
$A_{\text{net}}$		19.3***		10.77***
$A_{\text{net}} \times \text{NSC}$				6.1*

Significance levels: \*\*\* <0.001; \*\*<0.01; \*<0.05; ·<0.1.

## Figure legends

**Figure 1:** Changes in resprouting capacity as a function of species, water and light levels (a) and significant interactions (b, c) The species are *Quercus ilex* (Qi) and *Q. faginea* (Qf). The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table 1 for actual values). Sun or shade indicated whether the plants received either ambient, or 2-3% of ambient radiation, respectively. Values indicate mean and standard error.

**Figure 2:** Changes in total length, basal diameter and volume across species, light, water treatments and their interactions. Only significant interactions are included. The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table 1 for actual values). Sun or shade indicated whether the plants received either ambient, or 2-3% of ambient radiation, respectively. Values indicate mean and standard error.

**Figure 3.** Correlations between % resprouting and the three growth variables (total length, basal diameter and volume) in *Q. ilex* (left) and *Q. faginea* (right). Best fit lines indicate the results of simple regression rather than the full model and they are included only for visualization purposes. Dashed lines indicate that the full model was not significant. Each point is a treatment mean.

**Figure 4:** Effects of NSC and PLC over resprouting capacity in *Q. ilex* (left) and *Q. faginea* (right). Best fit lines indicate the results of simple regression rather than the full model and they are included only for visualization purposes. Dashed lines indicate that the full model was not significant. Each point is a treatment mean.

**Figure 5:** Correlates of growth across species. Photosynthesis ( $A_{\text{net}}$ ) correlated with length and volume in both species. NSC only correlated with diameter in *Q. ilex* and its interaction with  $A_{\text{net}}$  in volume of *Q. faginea*. Best-fit lines indicate the result of simple regression, not of the full model, and are included for visualization purposes only. Dotted lines indicate marginal relationships. Each point is a treatment mean.

Contrary to conventional wisdom here we show that carbohydrate reserves only play a minor role as drivers of resprouting in Mediterranean oaks. The probability of resprouting was mostly driven by the degree of antecedent hydraulic limitations and the growth of the resprouts depended largely on photosynthetic C uptake.

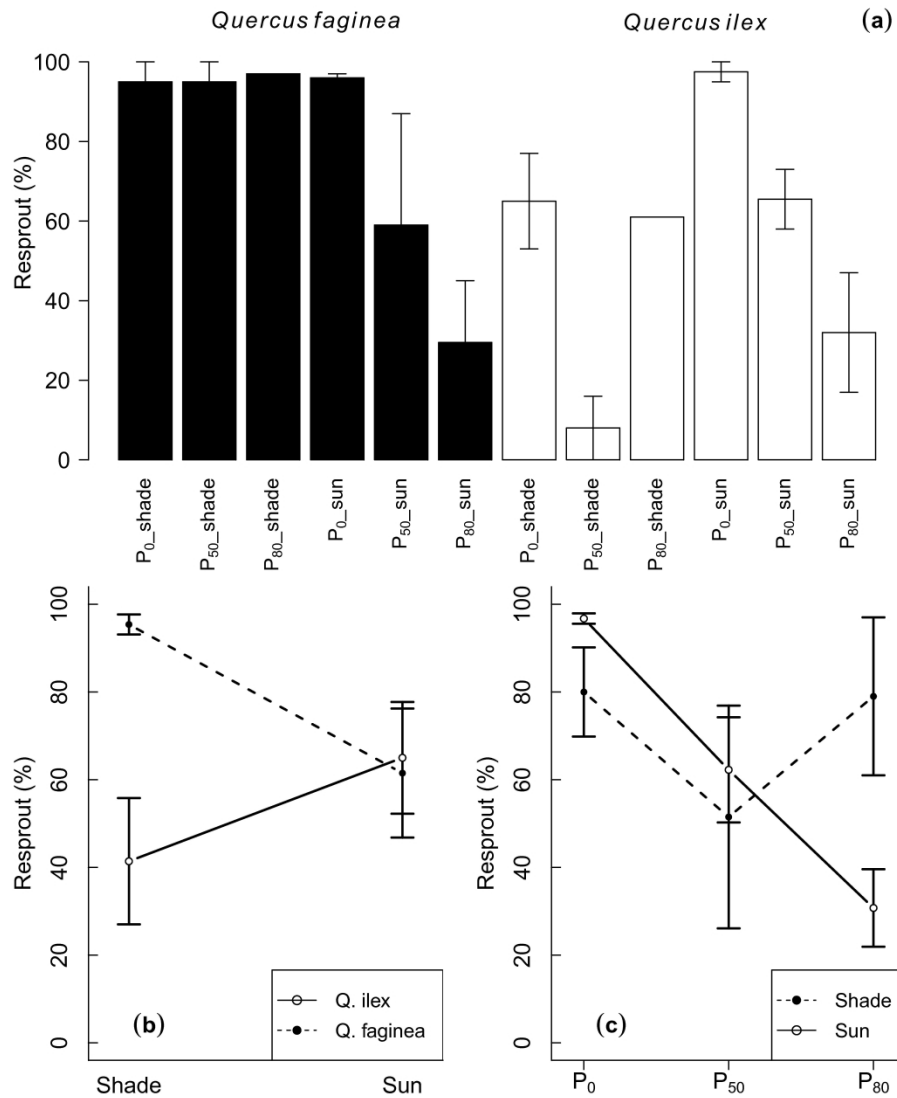


Figure 1: Changes in resprouting capacity as a function of species, water and light levels (a) and significant interactions (b, c) The species are *Quercus ilex* (Qi) and *Q. faginea* (Qf). The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table 1 for actual values). Sun or shade indicated whether the plants received either ambient, or 2–3% of ambient radiation, respectively. Values indicate mean and standard error.



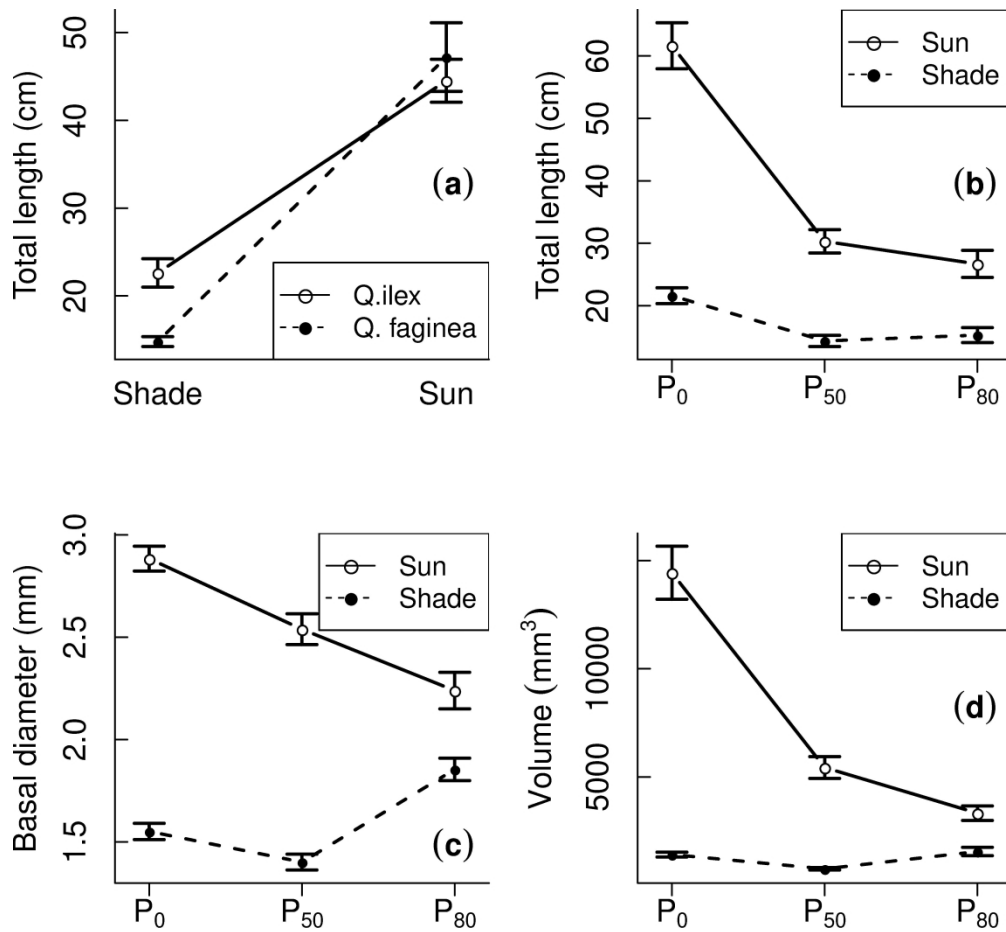


Figure 2: Changes in total length, basal diameter and volume across species, light, water treatments and their interactions. Only significant interactions are included. The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>88</sub>, but see Table 1 for actual values). Sun or shade indicated whether the plants received either ambient, or 4% of ambient radiation, respectively. Values indicate mean and standard error.

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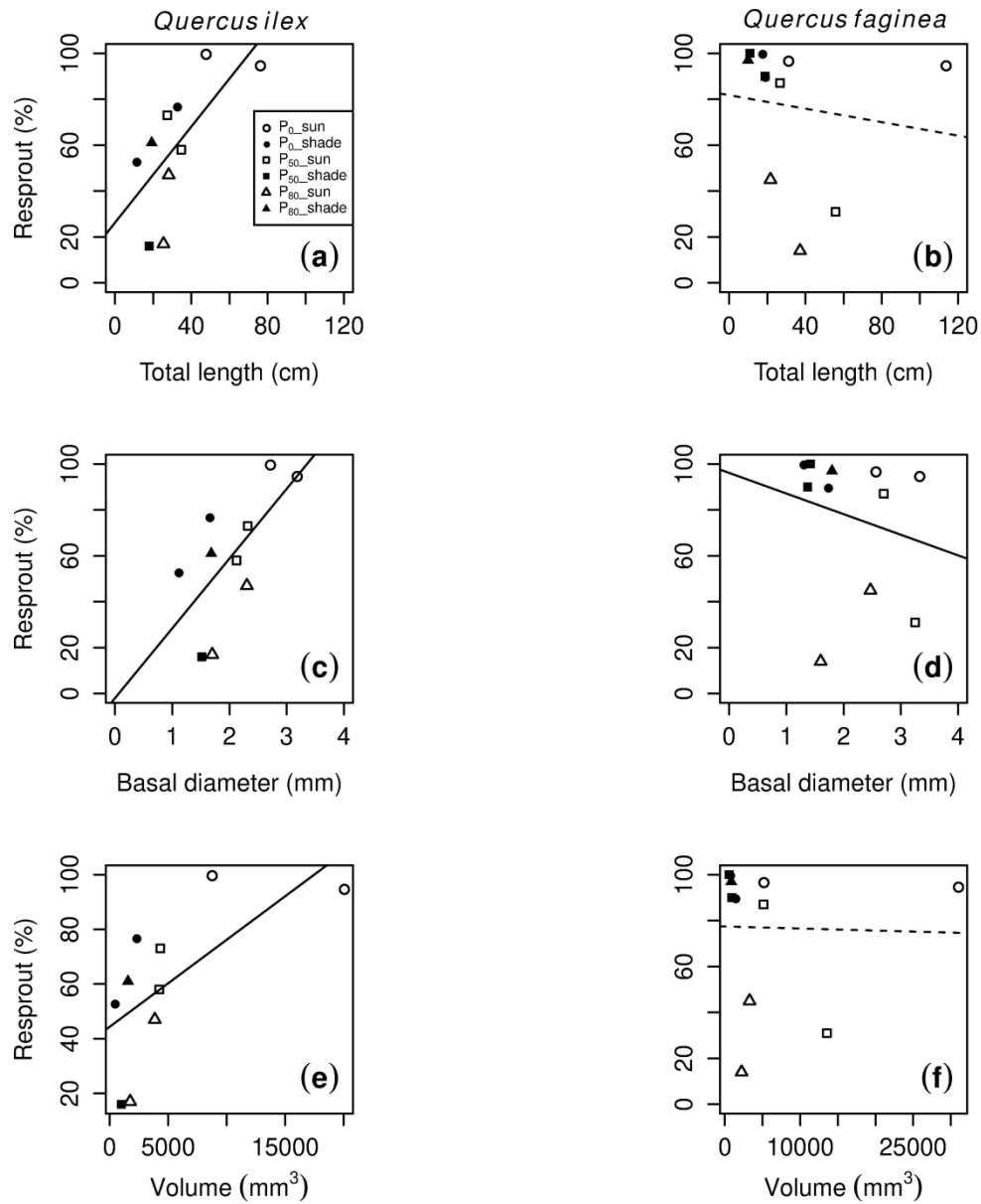


Figure 3. Correlations between % resprouting and the three growth variables (total length, basal diameter and volume) in *Q. ilex* (left) and *Q. faginea* (right). Best fit lines indicate the results of simple regression rather than the full model and they are included only for visualization purposes. Dashed lines indicate that the full model was not significant.

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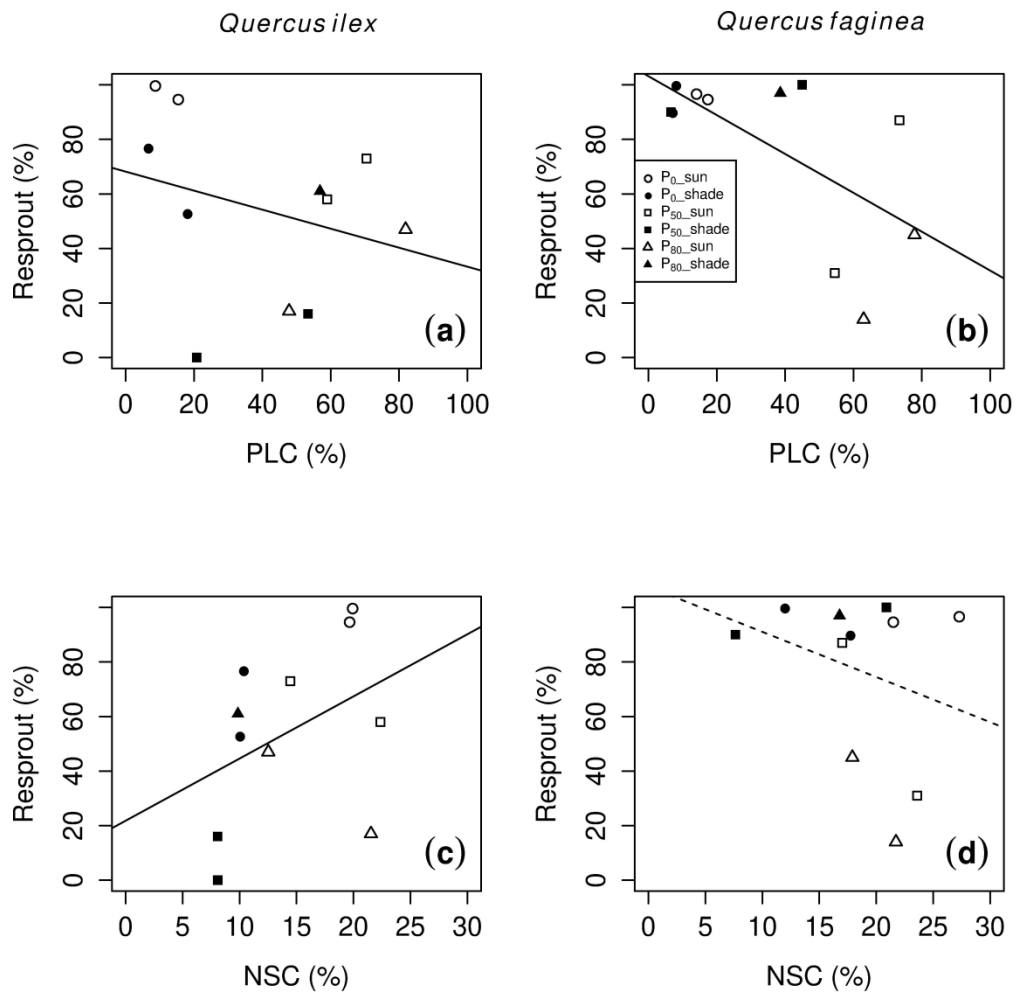


Figure 4: Effects of NSC and PLC over resprouting capacity in *Q. ilex* (left) and *Q. faginea* (right). Best fit lines indicate the results of simple regression rather than the full model and they are included only for visualization purposes. Dashed lines indicate that the full model was not significant.

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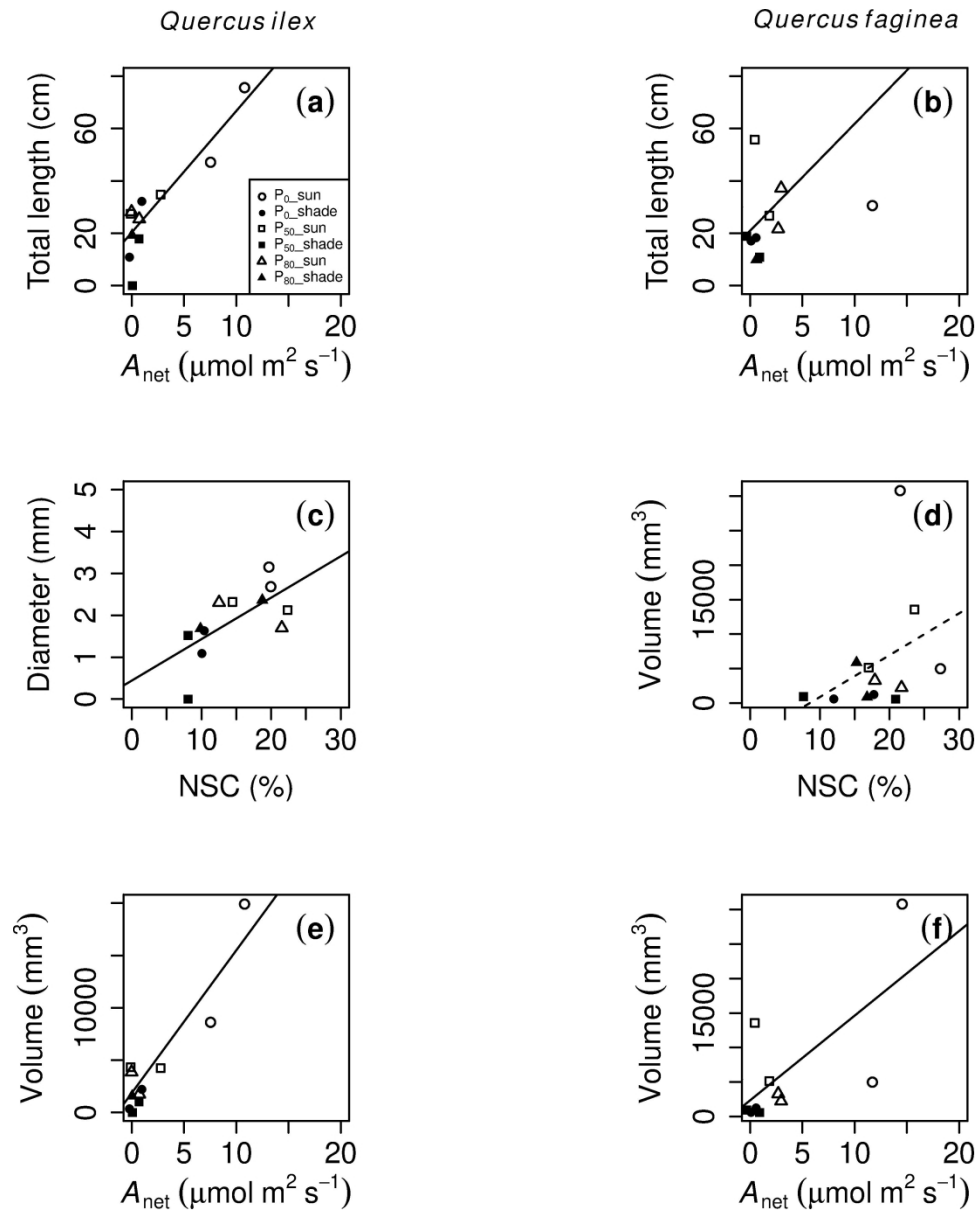


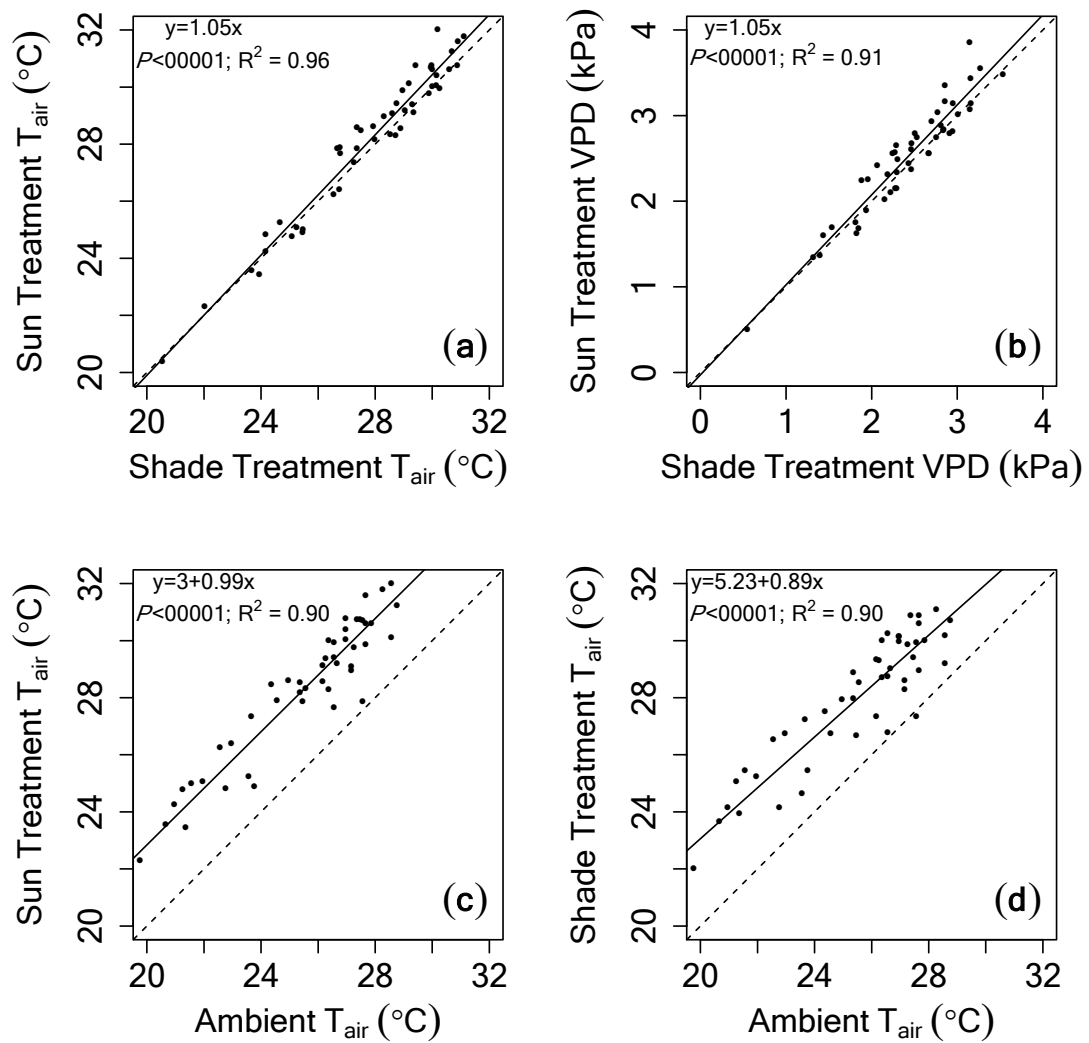
Figure 5: Correlates of growth across species. Photosynthesis ( $A_{\text{net}}$ ) correlated with length and volume in both species. NSC only correlated with diameter in *Q. ilex* and its interaction with  $A_{\text{net}}$  in volume of *Q. faginea*. Best-fit lines indicate the result of simple regression, not of the full model, and are included for visualization purposes only. Dotted lines indicate marginal relationships.

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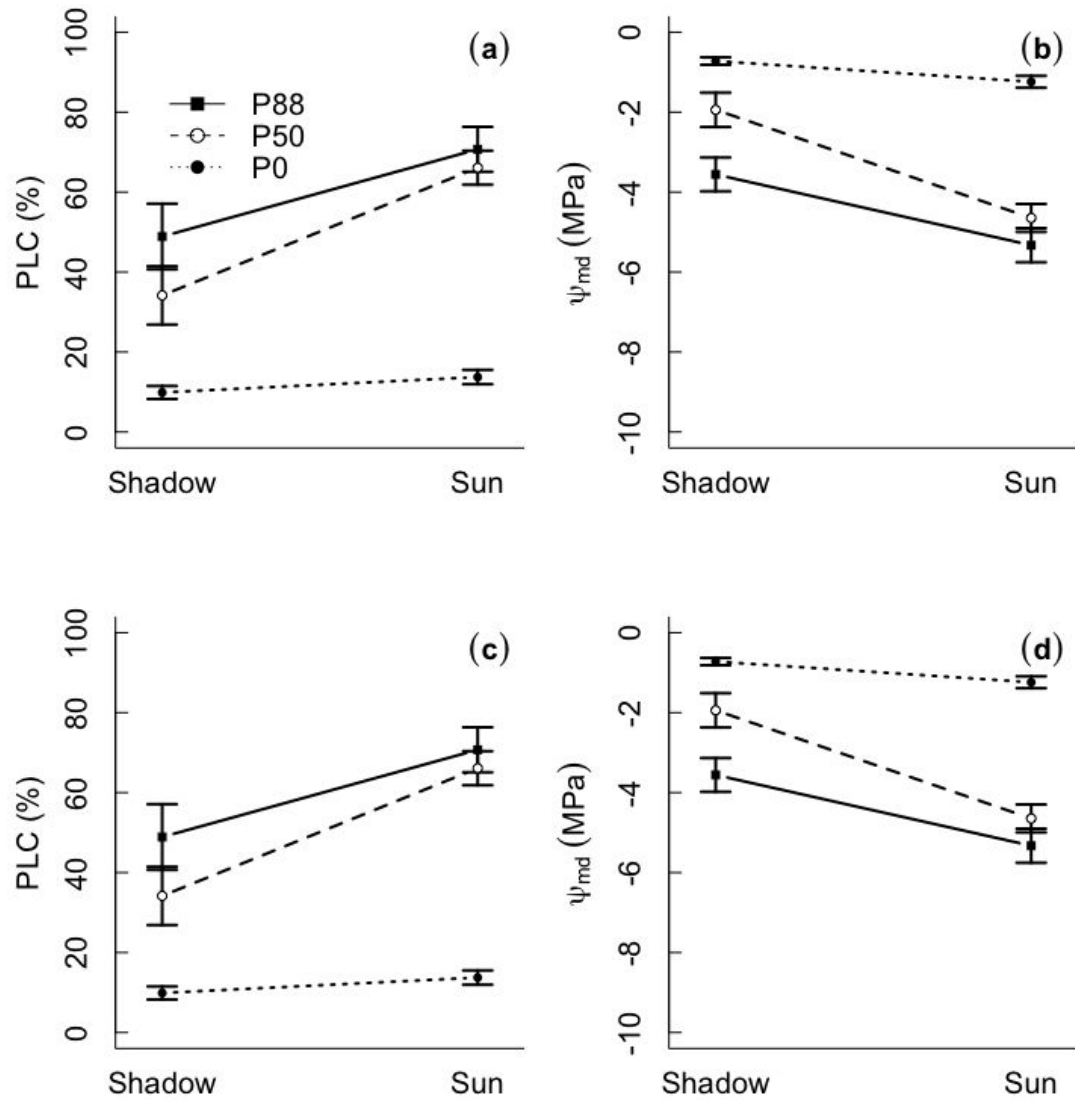
**Supporting information**

**Table S1:** results of linear mixed models analyses on differences across realized PLC values. Fixed factors were species, light and water treatments and their interactions and year was included as a random factor.

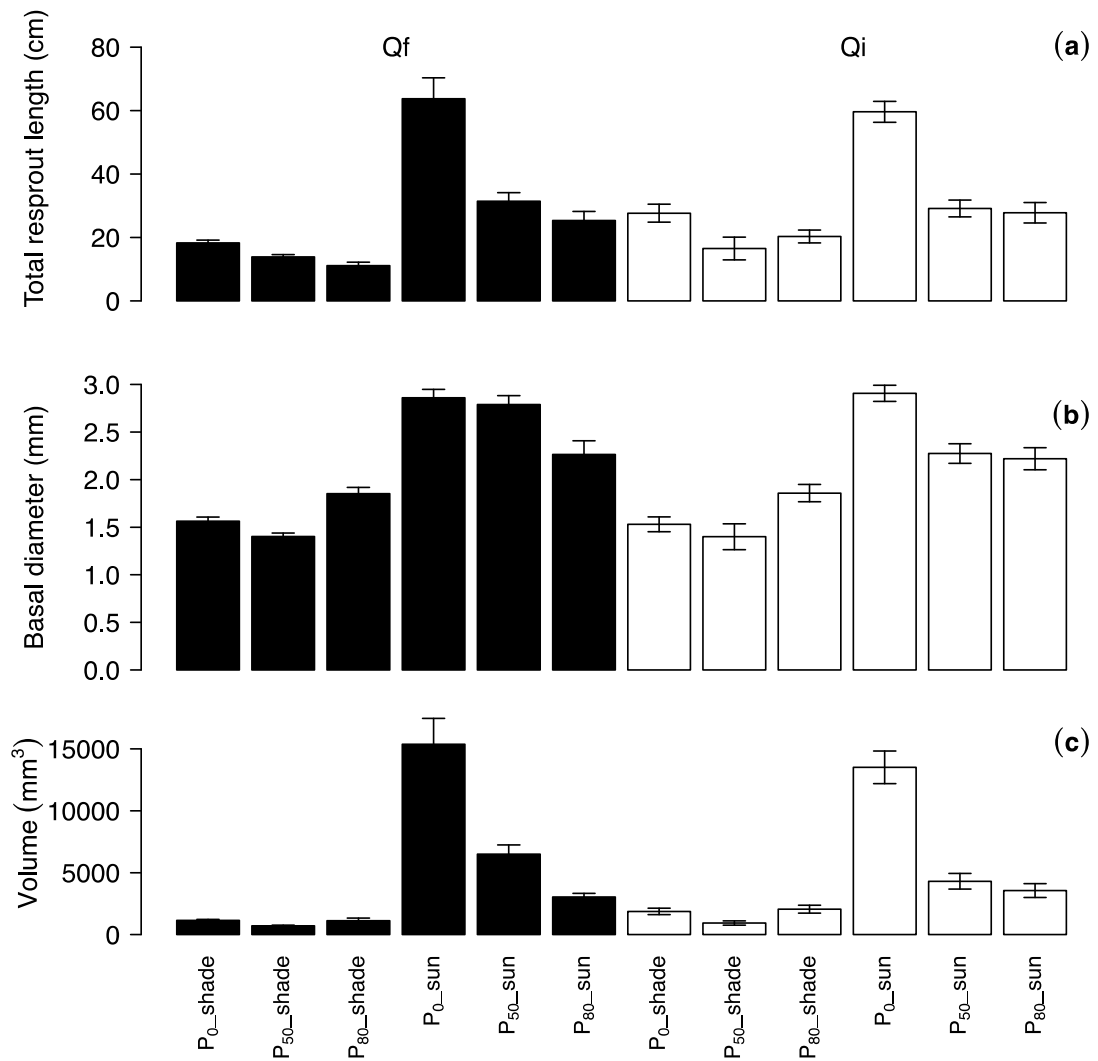
	$\chi^2$	P
Species	0.92	0.33597
Light	19.71	<0.0001***
Water	100.50	<0.0001***
Species $\times$ Light	2.81	0.09353
Species $\times$ Water	0.45	0.79661
Light $\times$ Water	8.56	0.01378*
Species $\times$ Light $\times$ Water	1.95	0.37602



**Figure S1:** Differences in mean daily air temperature ( $T_{\text{air}}$ ) and mean daily vapour pressure deficit (VPD) between sun and shade treatments (a, b) and in  $T_{\text{air}}$  between treatments and ambient (c,d). Data in the shelters were collected between June and July 2017 with a PCE-HT 71N (PCE Ibérica, Tobarra, Spain) logging automatically every 5 minutes. Ambient  $T_{\text{air}}$  data was downloaded from station number 9771C, from the Spanish Meteorology Agency ([www.aemet.es](http://www.aemet.es), 21<sup>st</sup> February 2020), which is placed inside the campus (<1 Km from the shelter).

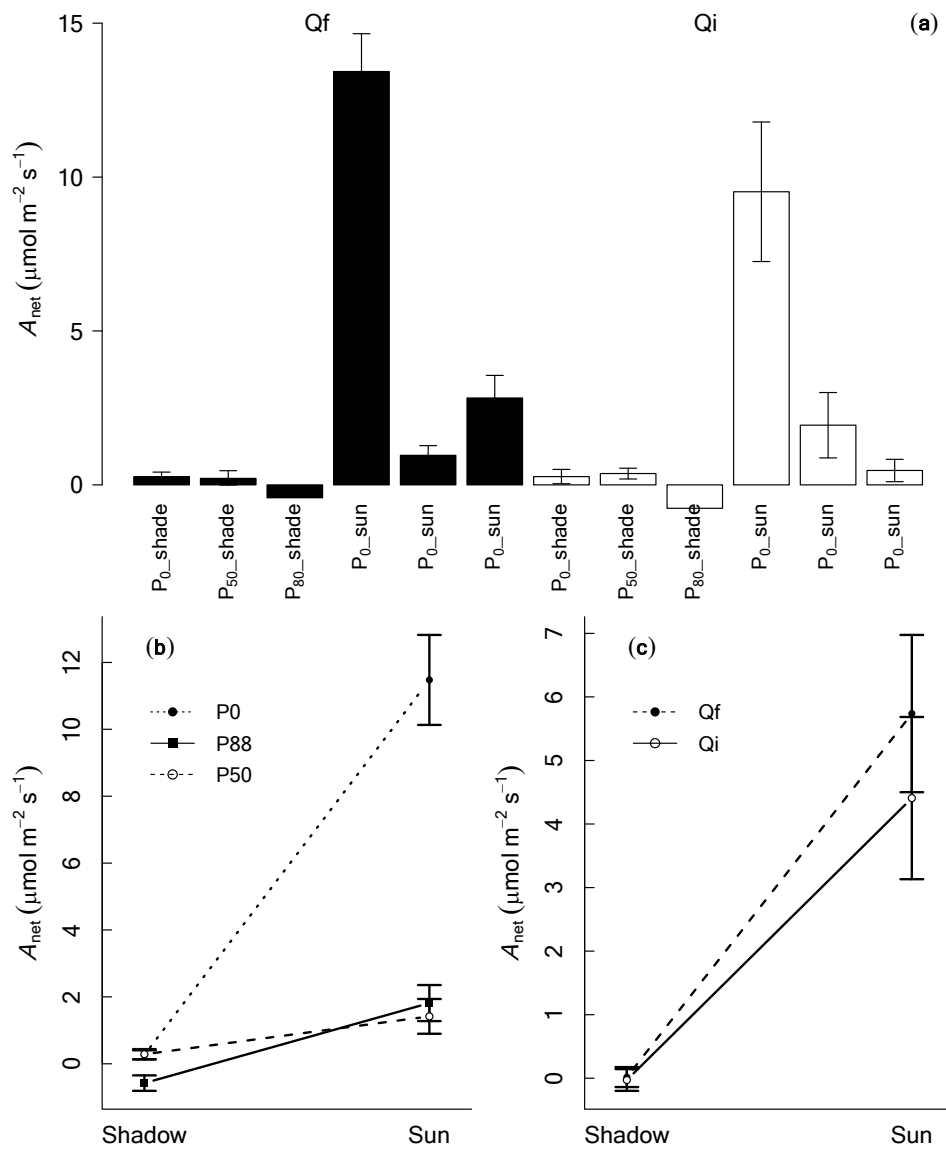


**Fig. S2:** Realized values of percent loss of hydraulic conductance (PLC) and midday water potential ( $\Psi_{md}$ ) at the time of inducing resprouting in *Q. ilex* (a,b) and *Q. faginea* (c,d). The target values were 0, 50 and 80% PLC ( $P_0$ ,  $P_{50}$  and  $P_{80}$ , respectively). Values indicate mean and standard error.



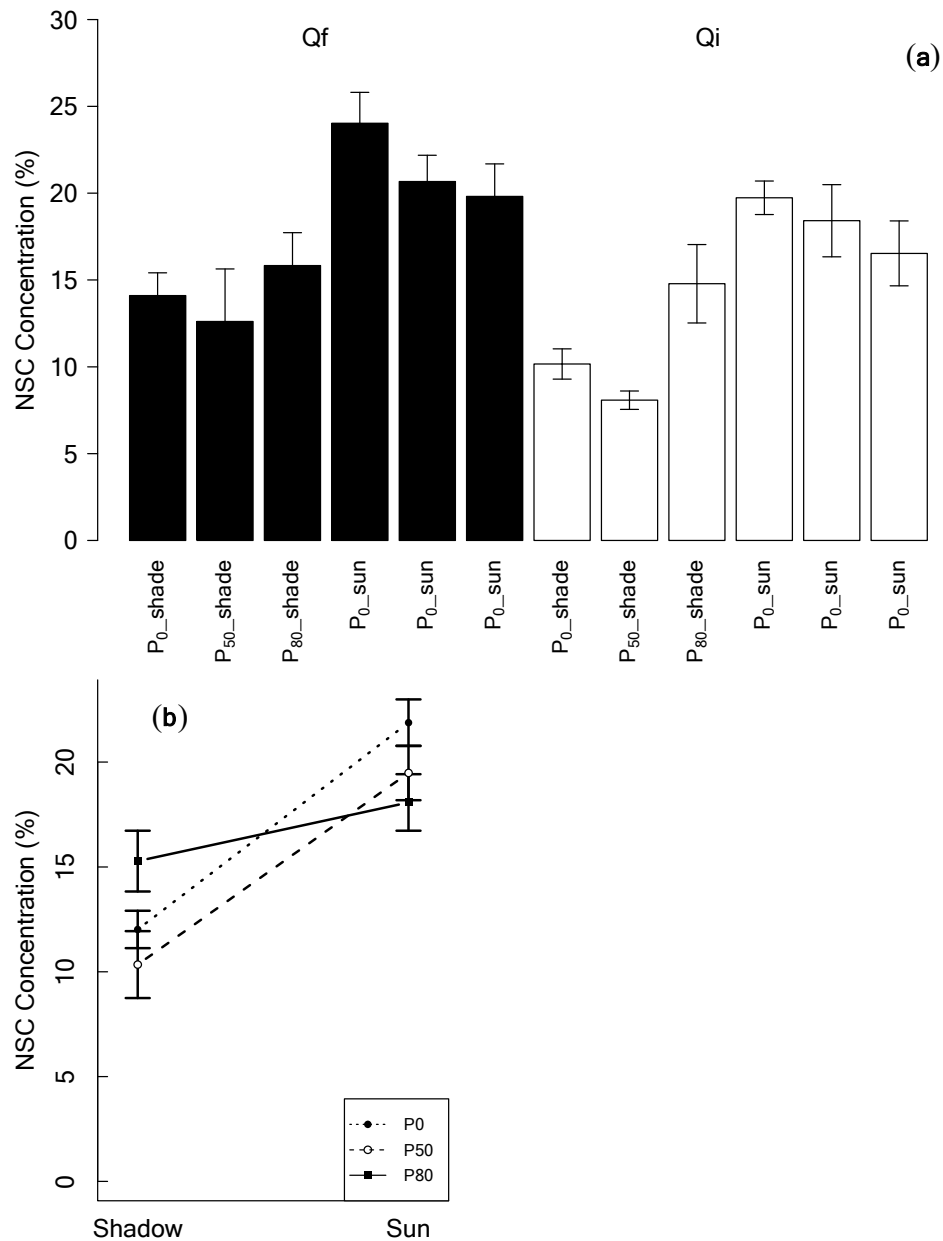
**Fig. S3:** Changes in resprouting growth as a function of species, water and light levels. The species are *Quercus ilex* (Qi) and *Q. faginea* (Qf). The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table 1 for actual values). Sun or shade indicate whether the plants received either ambient, or 4% of ambient radiation, respectively. Values indicate mean and standard error.



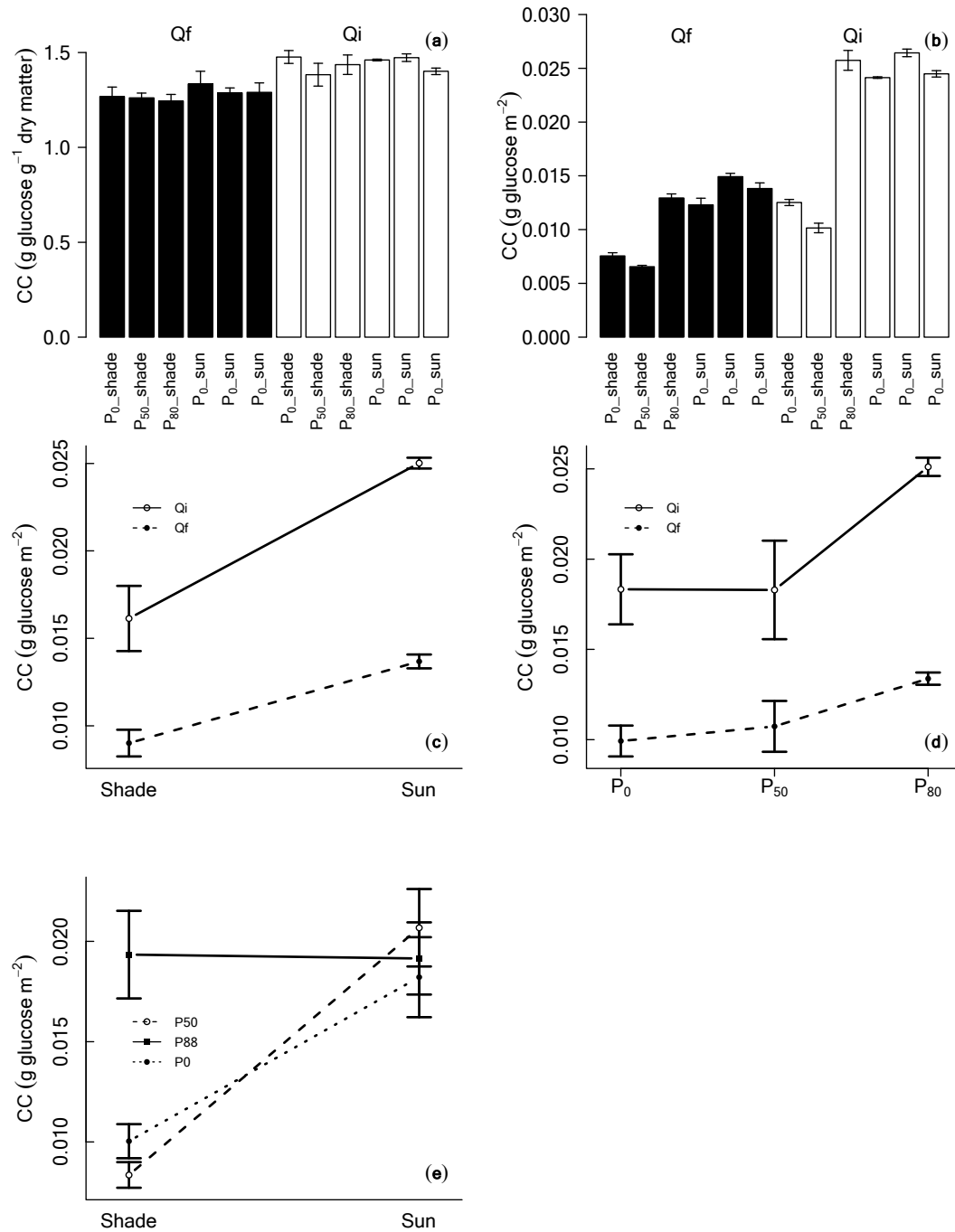


**Fig. S4:** Changes in net assimilation rate across species, light and water treatments.

To more clearly depict significant effects, we only include significant interactions (but note that the interaction in c is marginal). The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table1 for actual values). Sun or shade indicate whether the plants received either ambient, or 5% of ambient radiation, respectively. Values indicate mean and standard error.



**Fig. S5:** Changes in non structural carbohydrates (NSC) across species, light and water treatments. To more clearly depict significant effects, we only include significant interactions. The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table1 for actual values). Sun or shade indicate whether the plants received either ambient, or 5% of ambient radiation, respectively. Values indicate mean and standard error.



**Fig. S6:** Construction costs on a mass basis (a) and on a leaf area basis (b-e) as a function of experimental treatments. To more clearly depict significant effects, we only include significant interactions. The water levels refer to target levels of PLC (P<sub>0</sub>, P<sub>50</sub> or P<sub>80</sub>, but see Table 1 for actual values). Sun or shade indicate whether the

plants received either ambient, or 5% of ambient radiation, respectively. Values indicate mean and standard error.